THE DEVELOPMENT AND CLINICAL APPLICATION OF FIRST TRIMESTER CHORIONIC VILLUS SAMPLING FOR FETAL DIAGNOSIS

by

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AIMS OF THE THESIS

1. To critically review methods of prenatal diagnosis with special reference to chorionic villus sampling (Chapters 1-3).

2. To design suitable cannulae for transcervical chorionic villus sampling which fulfil certain specified criteria (Chapter 4.3).

3. To compare such cannulae with a widely used cannula in a randomised study examining factors influencing villus recovery and subsequent karyotyping (Chapters 4.5,4.6).

4. To investigate a method of transabdominal chorionic villus sampling and, in particular, the effect of placental site on villus recovery and comparing transabdominal with transcervical chorionic villus sampling methods (Chapters 5-6).

5. To assess methods of chromosomal analysis from chorionic villi using a direct method of karyotype preparation and comparing it to short term culture methods (Chapter 7).

6. To present a diagnostic series of patients in whom the methods of chorionic villus sampling developed were used for fetal diagnosis (Chapter 8).

7. To obtain previously unknown data on the spontaneous abortion rate in a cohort of first trimester pregnancies demonstrated to be viable by ultrasound examination and prospectively followed until at least 28 weeks gestation (Chapter 9).

ABSTRACT

THE DEVELOPMENT AND CLINICAL APPLICATION OF FIRST TRIMESTER CHORIONIC VILLUS SAMPLING FOR FETAL DIAGNOSIS

Two metal cannulae for transcervical chorionic villus sampling using a suction aspiration method were designed. In a randomised trial involving 200 transcervical villus samples taken with the two metal and one plastic cannula, the aluminium cannula performed best in terms of karyotype recovery and ease of insertion but placental site influenced the ability to recover villi for all cannulae.

A method of transabdominal chorionic villus sampling was adapted to existing equipment. It was compared to an improved method of transcervical chorionic villus sampling using the aluminium cannula, in fifty patients. The two methods were equally successful in obtaining villi but the transcervical method was significantly better at obtaining villi greater than 10 milligrams in weight. Placental position did not affect villus recovery with either sampling method.

Chorionic villi from twenty patients were prepared for karyotype analysis by a direct and short term culture method and no significant difference was found in the quality and quantity of metaphases obtained using either method. A further study involving twenty patients showed a variable response of any one villus sample to the methods suggesting an advantage to processing villi by more than one method.

One hundred patients had fetal diagnosis using the clinical and laboratory methods that were developed. Fetal diagnosis was possible in 97% of patients. The overall

spontaneous abortion rate was 5.7%. In a prospective study of 500 patients who had ultrasound evidence of a viable fetus below 12 weeks gestation, the spontaneous abortion rate was 2% overall.

In those women with a history of spontaneous abortion, the abortion rate increased tenfold. Spontaneous abortion at less than ten weeks was three times higher than that at greater than ten weeks gestation and this may have implications for the timing of first trimester chorionic villus sampling.

This work is dedicated to my wife Marilyn and our family.

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ABBREVIATIONS

cm	Centimetre
CVS	Chorionic villus sampling
Cu	Copper
°	Degrees
°C	Degrees centigrade
DNA	Deoxyribonucleic acid
gm	Gram
IU	International Units
Kg	Kilogram
min	Minute
ml	Millilitre
mg	Milligram
MHz	Megahertz
mm	Millimetres
ng	Nanogram
%	Percent
rpm	Revolutions per minute
RPMI	Rosewell Park Memorial Institute
sec	Second
S.D.	Standard deviation
X^2	Chi-square
µg	Microgram

CONTENTS

REVIEW OF FETAL ABNORMALITY

1.1 INTRODUCTION

A quarter of all perinatal deaths are due to congenital abnormalities according to the findings of the Scottish Perinatal Mortality Survey (McIlwaine et al 1979). In the past 50 years, the perinatal mortality rate has declined steadily as deaths from prematurity, birth asphyxia and infection have decreased (Nevin 1982). This fall has not been matched by a fall in the number of deaths from congenital abnormality and the proportion of perinatal deaths attributable to congenital abnormalities has risen in comparison to those from other causes (Nevin 1982).

Congenital abnormalities are present in 5% of liveborn children (Ferguson-Smith et al 1984). Data on admissions to paediatric hospitals in the United States of America has shown that from 21 - 27% are accounted for by congenital diseases or conditions with a genetic tendency (Day et al 1973; Hall et al 1978). Studies in the United Kingdom have shown that by age seven, 5% of all children suffer from some kind of impairment, and of these handicaps, 83% are congenital in origin (Wells 1978).

While there has been increasing understanding of the underlying causes of certain congenital disorders, coupled with advances in the clinical, cytogenetic, biochemical and molecular diagnostic techniques, only a limited number of congenital disorders can be treated. Prevention of congenital abnormality is possible through pre-pregnancy counselling in parents who suffer from conditions known to be associated

with a risk of fetal abnormality, but in many cases this will mean voluntary infertility or, in some cases, artifical insemination with donor semen for certain sex-linked disorders. Voluntary infertility has, for many couples, been the only choice when faced with the chance of bearing children with congenital abnormalities.

In the past twenty years, fetal diagnosis has offered parents the chance of selective abortion of an abnormal fetus and in fetal screening programmes, this has led to a 3 to 5% abortion rate in those screened (Galjaard 1980). For the majority, however, fetal diagnosis has been performed for reassurance of the presence of fetal normality and there is evidence that women at risk of having an abnormal fetus, who would previously have avoided conception, are now contemplating pregnancy because they know that screening for fetal abnormality can be offered and uncertainty about fetal normality removed (Weatherall 1985).

1.2 INCIDENCE OF MAJOR CONGENITAL DISORDERS

Recent endocrinological studies have demonstrated that approximately 50% of all conceptions fail (Miller et al 1980). Such failures may not be clinically obvious as a miscarriage and therefore are classed as a late period. Clinically apparent abortions occur in about 14% of pregnancies (Royal College of General Practitioners 1976). Chromosomal studies on abortus material demonstrates that the incidence of chromosomal anomalies decreases with the duration of pregnancy. In one large study of over 1,000 abortions, 49% were chromosomally abnormal at 8 to 11 weeks gestational age,

39% from 12 to 15 weeks, 18% from 16 to 19 weeks and 11% over 20 weeks (Warburton et al 1980).

Chromosomal aberrations were present in 9.5% of macerated stillbirths and 3.5% of non-macerated stillbirths. 5.1% of liveborn perinatal deaths had chromosomally abnormalities (Angell et al 1984).

External and environmental factors, such as teratogenic drugs, irradiation and transplacental bacterial and viral infections, account for 10% of congenital abnormalities (Kalter et al 1983).

The remaining abnormalities can be divided into three major categories of congenital disorder:-

A) Congenital malformations

B) Chromosomal aberrations

C) Mendelian (monogenic) disorders

A) Congenital Malformations

These constitute the greatest proportion of congenital abnormalities. Most are of unknown cause. Those, such as congenital heart defects, neural tube defects and oro-facial clefts may be caused by an interaction of genetic and external factors, as yet of unknown origin. The incidence of certain of these malformations, for example, neural tube defects, is affected by geographical and ethnic factors, the incidence rising from 1 to 2 per thousand in England to 4 to 8 per thousand in South Wales and Northern Ireland. It appears to be more prevalent in some areas of Celtic origin within the United Kingdom.

B) Chromosomal Aberrations

Data from the European Collaborative Study on the incidence of chromosomal aberrations, which was based on amniocentesis studies shows that numerical chromosomal aberrations such as Trisomies 21, 18, 13 and 47,XXY, increase with maternal age. Whereas the overall incidence of Trisomy is 2 per thousand newborns in women 24 years of age or less, it is 8 per thousand in the age group 35 to 39 years and up to 100 per thousand in women 45 years of age or older (Ferguson-Smith et al 1984). Certain other chromosomal aberrations do not show this maternal age effect e.g. 45,XO Turners Syndrome. The rates of chromosomal aberrations by maternal age groups are higher than liveborn rates, because a certain number of chromosomally abnormal fetuses abort between the gestational age at which amniocentesis would be performed and delivery. For example, the liveborn rate for Trisomy 21 is 30% less than the rate found in amniocentesis patients. The liveborn rate for Trisomy 18 is 68% less than the amniocentesis rate (Ferguson-Smith et al 1984).

In younger women, sporadic chromosomal disorders are associated with a recurrence risk of 1 to 2% (Mikkleson et al 1979).

Most chromosomal aberrations are sporadic non-disjunctions and rearrangements that occur during formation of the gamete. Fetal chromosomal aberrations are commoner where one parent has a balanced chromosomal rearrangement. This rearrangement is usually detected after chromosomal studies on those parents having repeated abortions, perinatal deaths or liveborn, with a chromosomally abnormal fetus. This can lead

in some cases to a high risk of fetal abnormality e.g. in the case of certain Robertsonian translocations in the mother, 15% of offspring will have Trisomy 21.

C) Mendelian Disorders

McKusick (1983) lists 3,500 different disorders of Mendelian inheritance. Of these, 1,637 have proven mutant genotypes, of which 934 are classified as autosomal dominant (57%), 588 as autosomal recessives (36%) and 115 as X-linked (7%).

The overall incidence of these monogenic disorders is probably 0.5 to 1.4% of liveborn births. Accurate estimations are difficult because of the complex techniques needed to be sure of the diagnosis of any one disorder and the variation in incidence of certain disorders among different ethnic groups (Galjaard 1980). Cystic fibrosis, for example, occurs in Caucasians in 1 in 2,500 births but has been estimated to be rare in Mongolian Chinese, at a rate of 1 in 90,000.

The prevention of congenital disorders without resorting to selective abortion is possible for a small number of conditions. Avoidance of environmental factors associated with congenital abnormalities such as mutagenic or teratogenic agents is possible. There is some, though not conclusive, evidence that periconceptional ingestion of multivitamins can reduce the incidence of neural tube defects as well as decrease the recurrence rate (Seller et al 1984; Smithells et al 1980).

For the chromosomal aberrations, apart from avoiding pregnancy within certain maternal age groups, no prevention short of fetal screening is possible. For the monogenic disorders, primary prevention is not possible, but if the facility for carrier detection existed for such disorders and was applied to large populations coupled with genetic counselling prior to reproduction, a major impact on the incidence of these conditions would happen.

1.3 THE IMPACT OF FETAL DIAGNOSIS ON THE BIRTH INCIDENCE OF CONGENITAL ABNORMALITIES

In the United States of America in 1970, there were fifty children with Tay Sachs disease born to Ashkenazy Jews. By 1982, with the widespread uptake of fetal diagnosis, there were nine new liveborn cases in this ethnic group (Kaback 1983).

With similar uptake of antenatal diagnosis within a high risk group e.g. homozygous beta-thalassaemia in Sardinia, the liveborn incidence of the condition fell three fold between 1977 and 1980 (Cao et al 1981).

For the more widespread chromosomal aberrations, the impact of fetal diagnostic techniques has not been so dramatic, reflecting the poorer uptake of antenatal diagnosis within the population. In theory, screening women over 35 years of age by amniocentesis for fetal Trisomy 21 could reduce the birth incidence of this defect by 35%, the actual figure in e.g. the Greater Glasgow Health Board area has been 6.5% in 1982, as only 28% of those at risk take up the offer of amniocentesis (Ferguson-Smith 1983). The amniocentesis

rate in women of 35 and over varies from 30% in 1984 in the West Midlands Health Authority area in England (Birmingham Maternity Hospital Annual Report 1984), to 85% in Denmark (Evans 1983). Such variation reflects not only public awareness that such a technique and service is available, but also the willingness of the medical profession to refer women for amniocentesis. When mass screening for a congenital abnormality is offered and accepted, the impact can be considerable. The birth incidence of neural tube defects fell by 72% in 1981 in the Greater Glasgow Health Board area with serum alphafetoprotein screening combined with subsequent ultrasound and amniocentesis confirmatory diagnosis (Ferguson-Smith 1983). This fall has occurred in other areas as well e.g. Mid Glamorgan in Wales had a fall in birth incidence of spina bifida of 8.4 per thousand births to 4.0 per thousand using serum alphafetoprotein screening (Laurence 1983).

Fetal diagnosis need not always be equated with abortion. Effective pre pregnancy prevention may occur for certain abnormalities, as has occurred for neural tube defects. Fetal therapy has a small place in antenatal care at present, but may expand in the future as the basis of the genetic defect in certain abnormalities begins to be understood.

FETAL DIAGNOSTIC TECHNIQUES (EXCLUDING CHORIONIC VILLUS SAMPLING)

2.1 INTRODUCTION

For the past thirty years, the possibility of fetal diagnosis has been realised and expanded to become an accepted and essential part of obstetric practice. Since the first reported amniocentesis in 1956 (Bevis 1956) in pregnancies affected by Rhesus isoimmunisation, the scope and range of fetal diagnostic techniques and indications has grown. Indeed, it was the availability of liquor from Rhesus affected pregnancies that gave Steele and Berg in 1966 the opportunity to develop fetal chromosomal analysis from human amniotic fluid (Steele et al 1966). From then on, the number of amniocenteses grew steadily until in 1983, it was estimated that between 70,000 and 140,000 amniocenteses were performed worldwide (Kaback 1983).

During this time, developments in ultrasound technology and imaging allowed invasive techniques such as amniocentesis and fetoscopy to become easier. As the image quality and precision of ultrasound improved, especially the development of real time grey scale ultrasonography, so the range of fetal abnormalities that could be diagnosed increased.

Certain procedures like fetoscopy and direct needle puncture of fetal vessels (cordocentesis) were concentrated in particular referral centres, so that the personnel involved became adept at them and consequently maternal and fetal complications were reduced. While it was recognised in the late 1960's that techniques should be developed that would permit very early fetal diagnosis (Warburton et al 1972),

it was not until reports appeared in 1982 of the successful use of chorionic villi obtained in the first trimester for the fetal diagnosis of haemoglobinopathies (Old et al 1982) and rapid fetal karyotype determination (Simoni et al 1983), that interest in using this tissue for first trimester fetal diagnosis was aroused.

The current methods used in fetal diagnosis are Amniocentesis, Ultrasound, Fetoscopy, Cordocentesis and Chorionic Villus Sampling.

2.2 AMNIOCENTESIS

Amniotic fluid and amniotic fluid cells obtained by transabdominal needle puncture can be used for the fetal diagnosis of chromosomal aberrations, fetal sexing, inborn errors of metabolism and neural tube defects.

Laboratory studies have shown that the lower limit of reliable amniotic fluid sampling for cytogenetic tests is 16 weeks gestation (Gosden 1983). Good tissue cultures can be obtained from 10 mls of amniotic fluid. Results usually take three weeks to obtain, although with hormone enriched media, the time can be reduced to 10 to 12 days. In many cases therefore, a diagnosis of fetal abnormality will not be obtained until 18 weeks gestation at the earliest.

There have been several large studies on the safety of amniocentesis. A study from the United States of America had found no difference in fetal loss rates between cases and controls, but there may have been bias in the selection of the controls (N.I.C.H.D. 1976). A Canadian study demonstrated that the fetal loss rate between controls and

those having an amniocentesis was the same (Medical Research Council of Canada Report 1977). In contrast, a British study (Medical Research Council Report 1978) found a 1.3% increased spontaneous abortion rate in the amniocentesis group compared with controls. In a randomised study from Denmark, which examined women at low risk of fetal abnormality, the excess abortion rate in the amniocentesis group was 1% (Tabor et al 1986). This report also found an increased rate of transient respiratory difficulty in babies from the amniocentesis group compared with controls as had also been noted in the British study (Medical Research Council Report 1978). The Danish study did not however confirm the increased risk of orthopaedic abnormalities found in the British study. Factors affecting the risk of abortion in Tabor's study included transplacental amniocentesis, but there was no increase in intrauterine growth retardation or prematurity.

One factor not mentioned in the Danish study was the influence of the needle size used for the amniocentesis, as a factor in causing spontaneous abortion. Whereas they had reported that an 18 gauge needle was used for all amniocenteses, the usual practice in the United Kingdom was for a smaller 21 or 22 gauge needle. Data from two surveys (N.I.C.H.D. 1976; Medical Research Council of Canada Report 1977) supported the view that the use of needles larger than 19 gauge was associated with an increased abortion rate. Perhaps the use of the larger needle in the Danish group's study led to a greater abortion rate than would have occurred had a smaller needle been used.

This caveat aside, the Danish randomised study allowed a prediction of miscarriage from amniocentesis to be reliably quoted, thus ending the uncertainty caused previously by biased studies.

2.3 ULTRASOUND

With the advent of real time high resolution ultrasound equipment, requiring few operating manoeuvres to obtain good quality ultrasound images, the number of ultrasound procedures performed on pregnant women has markedly increased. A Royal College of Obstetricians and Gynaecologists Working Party on Routine Ultrasound in Pregnancy (Royal College of Obstetricians and Gynaecologists Report 1984) estimated that during 1983 up to 75% of all pregnant women in England had ultrasonography performed compared with less than 10% in 1972. Rates in Scotland were similar and in Northern Ireland nearly 100% of all pregnant women had ultrasonography in 1983.

Early reports on the diagnosis of fetal abnormality by ultrasound concentrated on the diagnosis of anencephaly and spina bifida. In a centre specifically performing a fetal anomaly scanning service, 211 cranio spinal defects were detected, out of a population of 1,473 referred because of high risk (Campbell 1984).

The sensitivity of ultrasound in detecting neural tube defects improved from 33% to 80% in another report from a non-specialised centre in a 10 year period (Roberts et al 1983).

Such success in a population at high risk for a fetal disorder, such as spina bifida, is not matched in studies on the low risk population, when screening for fetal disorders. Persson et al, in 1983, reporting on their long term experience of routine ultrasound screening in pregnancy, detected only 12 of 50 fetal malformations at 17 weeks gestation. The majority (38 of 50) were detected at 33 weeks gestation, a stage at which termination of the pregnancy would not have been possible if desired. Similarly, Eik-Nes et al, in 1984, reporting on one of the few randomised studies examining the effectiveness of routine ultrasound screening,showed no statistical difference in perinatal mortality or morbidity rates between the routine and the non-routine scanned groups.

As the frequency of most major structural defects is low, about 1 in 1,000 births, any one ultrasonographer will spend many thousands of hours performing detailed scans on many women in order to pick up one abnormality.

Routine mass screening for fetal abnormality, using ultrasound in populations at low risk, is likely to be ineffective as long as the rate limiting factor is machine quality, and operator experience and concentration.

2.4 FETOSCOPY

Fetoscopy is a method of direct fetal visualisation in which a small 2 mm diameter endoscope is passed trans-abdominally through the anterior wall of the uterus and into the amniotic sac. Originally used for the diagnosis of neural tube defects, its use was extended to umbilical cord

blood sampling and tissue biopsy from the fetus. Its use in the management of Rhesus isoimmunisation has extended to fetal therapy by direct intravascular transfusion in severely affected fetuses. It has been used extensively for the diagnosis of Haemoglobinopathies and Haemophilia, and in the rapid karyotyping of fetal blood in non immune Hydrops and intrauterine growth retardation, when a fetal chromosomal abnormality is suspected (Nicolaides et al 1985). Fetoscopy is associated with a 2 - 5% spontaneous miscarriage rate and an 8 - 10% incidence of premature labour. Four to five per cent of women having a fetoscopy will have a persistent leak of amniotic fluid (Rodeck 1983).

These high complication rates restrict the indications for the procedure. The usefulness of fetoscopy has now declined to be replaced by cordocentesis and chorionic villus sampling.

2.5 CORDOCENTESIS

With the improvement in resolution of ultrasound equipment, real time visualisation and guidance of needles into the umbilical cord has become possible. This technique, named 'Cordocentesis', has largely replaced fetoscopy. Apart from obtaining fetal blood for use in the diagnosis of chromosomal, biochemical and blood disorders, its use has also been extended to fetal therapy, in the case of Rhesus isoimmunisation (de Crespigny et al 1985).

It has also been used in studies of fetal oxygenation and in acid base studies of the growth retarded fetus (Soothill et al 1986 a; Soothill et al 1986 b).

In the United Kingdom, its use has largely been restricted to one or two centres and estimations of fetal loss from large series of cordocenteses can be as low as 7 out of 562 cases (Daffos et al 1985). The fetal losses, where the indication is Rhesus isoimmunisation or fetal hypoxia, may be greater.

CHORIONIC VILLUS SAMPLING

3.1 INTRODUCTION

The techniques of amniocentesis, ultrasound, fetoscopy or cordocentesis can only be used for the prenatal diagnosis of fetal abnormality in the second trimester of pregnancy. The earliest gestational age used for amniocentesis for cytogenetic purposes is 16 weeks. Data from the West Midlands Amniocentesis Audit 1985 shows a lag time of 10 to 22 days for the karyotype result leading to an average gestational age at termination of pregnancy for karyotype abnormality at 19 weeks 2 days (range 17 weeks 5 days to 23 weeks 5 days).

At such gestations, the majority of pregnant women will have felt fetal movement. Uterine size will be obvious, not only to the woman herself but also to her family and friends. If termination of pregnancy is undertaken, because of fetal abnormality, the physical and emotional complications are considerable in comparison to first trimester termination of pregnancy. In two studies, depression and guilt were long term effects of second trimester termination of pregnancy for reasons of fetal abnormality (Donnai *et al* 1981; Blumberg *et al* 1975).

Midtrimester abortion using prostaglandins as the abortificient agent has a complication rate of 24 per 100 abortions compared to that of first trimester abortions, in which the equivalent rate is 6 per 100 abortions (Grimes *et al* 1979). In one study, the risks of maternal death rose from 0.6 per 100,000 first trimester abortions to 15.7 per 100,000 abortions carried out between 16 to 20 weeks (Binkin 1986).

The problems associated with delayed diagnosis and late termination of pregnancy had been recognised as drawbacks of amniocentesis and had led to unsuccessful attempts at first trimester amniocentesis via a vaginal approach (Scrimgeour 1973).

The possibility of using tissue other than amniotic fluid for earlier prenatal diagnosis was first explored in Scandinavia in the late 1960's.

3.2 THE EMBRYOLOGICAL DEVELOPMENT OF CHORIONIC VILLI

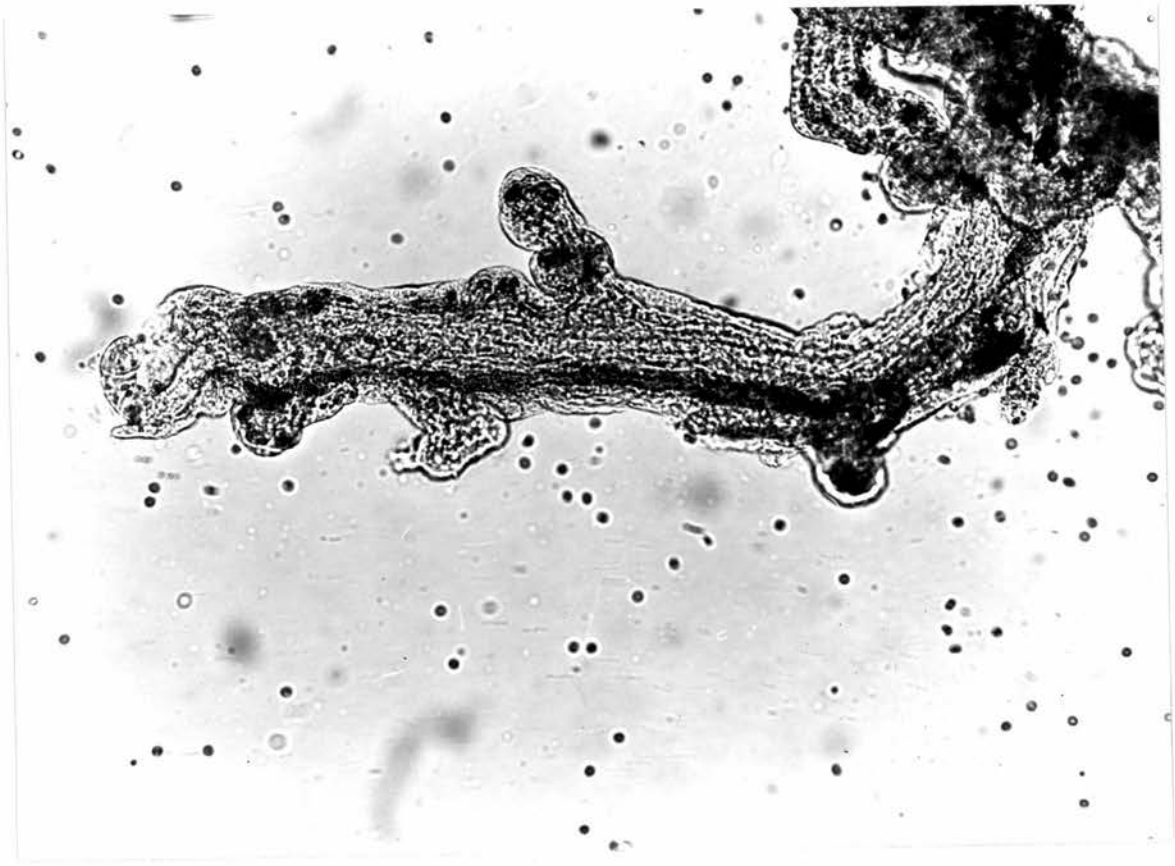
After fertilisation in the Fallopian tube, the ovum enters the uterine cavity as a dividing ball of cells (the morula). After shedding its outer covering (the zona pellucida) it is termed a blastocyst. The outer layer of the blastocyst (the trophoblastic cell mass) will form the outer embryonic membrane (the chorion) and placenta. The inner layer of the blastocyst will develop into the embryo itself as well as the amnion and yolk sac. Normally all cells of the blastocyst are diploid (46 chromosomes) and of fetal origin.

The chorion is composed of an outer layer of trophoblast and an inner mesodermal layer containing blood vessels. The trophoblast cells invade the endometrium and differentiate into two layers. The inner layer consists of mononuclear cytotrophoblastic cells with well defined membranes. The outer layer of syncytiotrophoblast, is formed by fusion of cytotrophoblastic cells and there are no obvious cell membranes visible. DNA synthesis and mitotic activity occur in the nuclei of the cytotrophoblastic cells but not in the

syncytiotrophoblast. Between days 13 and 14 after fertilisation, the cytotrophoblast proliferates into the syncytiotrophoblast to form clumps termed primary chorionic villi. By the time these acquire connective tissue cover (day 15), they are termed secondary villi. They eventually cover the entire surface of the chorion. When capillaries develop in the connective tissue cores of the villi they are termed tertiary villi. All three types of villi can be present at the same time. The cytotrophoblastic extension from the villi which penetrate the syncytiotrophoblast and join, form the cytotrophoblastic shell which anchors the chorionic sac to the maternal endometrium.

Up to eight weeks gestation, the entire chorionic sac is covered with villi but as the sac grows from the implantation site, the blood vessels in the decidua capsularis (the area overlying the conceptus) are compressed and degenerate, the villi disappear and the region becomes smooth (termed the chorion laeve at that stage). At around this time, villi in the decidua basalis (the area underlying the conceptus) increase and arborise to form a thick layer termed the chorion frondosum (the fetal component of the placenta). The decidua basalis forms the maternal component (decidual plate). As the villi invade the decidua basalis, they leave decidual tissue wedges called placental septa which divide the fetal part of the placenta into 10 to 38 cotyledons, each of which contain two or more stem villi.

A single villus examined under a low power microscope has the appearance noted in figure 1. The outer covering is two layered with an outer syncytiotrophoblastic and an inner



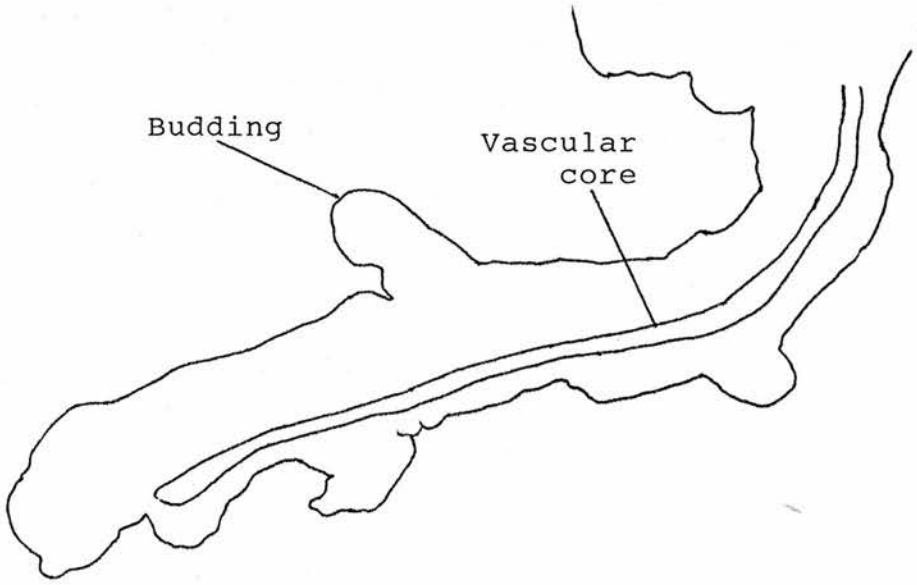


FIGURE 1: A SINGLE VILLUS SHOWING 'BUDDING' SPROUTS AND VASCULAR CORE (x500)

cytotrophoblastic layer. It is within the inner layer that mitotic activity occurs and it is these cells that are used in the direct preparation technique of cytogenetic analysis (Chapter 7).

The chorion frondosum is 3 to 6 mms thick and can be identified in the pregnant woman using grey scale ultrasonography. It is the villi of this area that is sampled in chorionic villus sampling. The chorion mass is about 5 to 80 gms at 8 to 12 weeks gestation (Rushton 1987), and typical diagnostic samples of 10 to 20 mgs will remove from 0.1 to 0.4% of the total villus mass.

3.3 TRANSCERVICAL CHORIONIC VILLUS SAMPLING TECHNIQUES

A) Endoscopic (Direct Vision) Techniques

In 1968, Mohr in Denmark had proposed taking biopsies of the chorion of early pregnancies for fetal study using a transvaginal hysteroscopic approach. Hahnemann and Mohr in 1968, presented the first report on this work and Hahnemann reviewed all their work from 1968 to 1974, in a report on a total of 95 cases (Hahnemann 1974).

The sampling instrument consisted of a 6 mm diameter hysteroscope with a forward visual angle of 60°. Close to the tip of the instrument was a 4 x 4 mm opening into which the tissue to be sampled was sucked by the application of a partial vacuum. A cylindrical knife housed within the cannula was then advanced and rotated cutting the tissue for removal. Ninety five women from 8 to 14 weeks gestation scheduled for termination of pregnancy had such a procedure attempted. All but 28 had it performed under general

anaesthetic prior to the termination. A satisfactory biopsy was obtained in 50 women. In 24 patients, in whom fetal tissue was obtained after the termination, culture of such tissue matched the culture of the sample. Of 24 patients, who had sampling performed without anaesthesia, as out patients, ten had complications of the procedure 'inconsistent with continuing pregnancy'. Although Hahnemann concluded that the procedure was feasible as a method of obtaining tissue sufficient for prenatal diagnosis in the first trimester, the hazards of the procedure to the fetus were considerable.

Kullander and Sandahl, in Sweden (1973) also experimented with chorionic villus sampling using a direct vision approach. Their device, which they termed an endocervicoscope was 5 mms in diameter, with forward view incorporating a biopsy forceps which could grasp the villi and avulse it. They used this device on 39 patients prior to termination of pregnancy, some under general anaesthesia and some without (precise numbers were not given). Successful culture of villi for chromosomal analysis was possible in 14 of 25 first trimester patients, and 7 of 14 second trimester patients. Interestingly, 19 patients had a delay of between 7 and 43 days from sampling to termination and there was no evidence of a spontaneous abortion in these patients, although precise ascertainment of fetal viability was not performed, ultra-sonography not being routinely available at that time.

These two Scandinavian studies proved that chorionic villus sampling was possible, that karyotyping of the fetus could be done from the samples obtained and in the short follow up of a small number of patients, immediate abortion did not occur.

These studies did not lead to other published work in this technique of prenatal diagnosis until 1982.

During the 1970's, the research and applied uses of amniotic fluid coupled with the relative ease of obtaining it concentrated efforts into amniocentesis for fetal diagnosis.

In 1982, a report of first trimester chorion biopsy using an endoscopic technique (and later an ultrasound guided technique) appeared from the Soviet Union (Kazy et al 1982). In 55 cases prior to termination of pregnancy chorion was obtained using an endoscopic technique. A 1.7 mm fetoscope was passed through the cervix into the uterus until the chorion frondosum was seen. Tissue was removed using biopsy forceps included with the fetoscopic unit. One hundred and ten cases had chorion biopsies attempted using a flexible forceps with an outer diameter of 2 mms, which was inserted and guided to the chorion by means of continuous real time ultrasound guidance. Whereas the majority of the 165 cases reported by this group had villus sampling performed for the analysis of enzymatic activity, 26 patients at risk of sex linked conditions had sampling performed for fetal sexing. Thirteen of these had their pregnancies terminated but 13 continued without incident, and the report contained details of the delivery weight of 11 patients (2 being undelivered at the time of the report).

This was the first report of successful chorionic villus sampling using ultrasound to guide the sampling instrument and also to give details of the follow up of diagnostic cases to delivery.

In 1982, another report on direct vision chorionic villus sampling appeared, in which fetal sexing was the indication for the procedure. All patients were sampled under general anaesthetic before termination of pregnancy. A 2.2 mm fetoscope was used transcervically but a new feature was the instillation of several millilitres of warmed isotonic saline to facilitate separation of the decidua capsularis from the decidua parietalis to aid visualisation of the chorion (Gosden et al 1982).

Using a similar fetoscopic system, combined with the instillation of warmed isotonic saline, Gustavii reported on 100 diagnostic cases (Gustavii 1984 a). His fetoscope was 1.7 mms in diameter with a specially designed channel 3.0 by 4.7 mms in outer diameter containing two attachments, one for the infusion of the warmed isotonic saline and the other for the biopsy forceps.

By 1984, Gustavii had the world's largest experience of diagnostic chorionic villus sampling using an endoscopic technique. In 96 of his 100 diagnostic cases, laboratory analysis of the villi was successful. There was a 7% spontaneous miscarriage rate in his reported series. All sampling was performed without analgesia and he used ultrasound to aid in the guidance of the instrument to the sampling site.

Although vascularised villi could be identified and removed by this method, the length of time for intrauterine instrumentation in some cases was over 15 minutes (Personal Observation at the University of Lund, October 1984).

By 1985, Gustavii had abandoned this method of diagnostic chorionic villus sampling in favour of trans-abdominal chorionic villus sampling and by the end of that year no major centre in the world was performing chorionic villus sampling using an endoscopic technique.

B) Biopsy Forceps Combined with Ultrasound

As previously stated in Section 3.3 A, Kazy in 1982 had reported on a series of experimental cases using this approach. Dumez, by 1984, could report on the results of 120 diagnostic cases (Dumez et al 1985). He used a rigid forceps of 2 mms in diameter and 20 cms long (originally designed for paediatric laryngoscopy) combined with real time ultrasound guidance. A tenaculum applied to the cervix allowed straightening of the utero-cervical canal as well as counter traction at insertion of the forceps. These workers usually obtained villi in up to 3 sampling attempts. Of the 120 diagnostic cases (the majority for fetal sexing and DNA analysis), there were 41 terminations of pregnancy and 6 spontaneous abortions (a rate of 7.6%).

(Such was the influence of this method in France, that at the time of the Third International Conference on Chorionic Villus Sampling and Early Prenatal Diagnosis in December 1986, this was the preferred method of sampling of French and Belgian workers, but of no other centre outside these countries).

METHODS OF TRANSCERVICAL CHORIONIC VILLUS SAMPLING USING SUCTION ASPIRATION

4.1 INTRODUCTION - Review of Published Papers 1975 to 1984

The technique of transcervical chorionic villus sampling using suction aspiration owes its origins to Chinese workers, who in 1975 published the results of their 5 year experience of transcervical chorionic villus sampling using a metal cannula (Anshan 1975). The paper examined fetal sex prediction by the identification of sex chromatin from chorionic villi cells. One hundred first trimester pregnancies were studied. The apparatus used to obtain villi was a 3 mm diameter calibrated metal cannula with an inner fine suction tube, with a blunt tip which protruded about 1 cm from the end of the cannula. The inner cannula was connected to a 5 ml syringe. The cannula was inserted through the cervix and the proposed sampling site chosen was the part of the anterior or posterior uterine wall that was the softer. Once the direction of the cannula was chosen, it was introduced through the utero cervical canal, to a depth of 6 to 9 cms from the external os and then when soft resistance was felt, further introduction was stopped. The inner tube was then pushed forward 0.5 to 1.0 cm and aspiration performed. The results of their 100 diagnostic cases are summarised in Table 1. Of 66 full term deliveries, that they reported, there were no antepartum or post partum problems in any case.

In 1975, a paper also appeared by Rhine *et al*, in which first trimester karyotype diagnosis was attempted in tissue sampled from the endocervical canal. Their hypothesis was

TABLE 1

OUTCOME OF 100 PREGNANCIES REPORTED FROM ANSHAN 1975

Prediction		Abortions		Delivery	
Sex	No. of cases	Induced	Spontaneous	Male	Female
Male	53	1	2	47	3
Female	46	29	1	3	13
Unknown	1		1		

that there existed an area of trophoblastic cell accumulation at the internal os of the cervix. Using an instrument they named the Antenatal Cell Extractor (ACE for short), they successfully obtained tissue from 17 of 21 patients prior to first trimester termination of pregnancy. The ACE was a 3.5 mm diameter plastic tube with a moveable bulbous tip which occluded the lumen of the tube during its passage through the cervical mucous into the region of the internal os. A small quantity of saline was injected and aspirated and examined for the presence of trophoblast cells. In 17 patients, cells were karyotyped in 11 and in 4 they were said to be different from that of the mother using Y body fluorescence.

In a further series reported in 1979, Rhine and Milunsky took samples from 53 patients in the first trimester prior to termination of pregnancy. Fetal chromosomes were demonstrated in 26 (49%). This report which formed part of a book entitled Genetic Disorders and the Fetus, made a case for first trimester fetal diagnosis as an alternative to amniocentesis and in particular for aspiration of trophoblast for chromosomal analysis.

Further interest in first trimester diagnosis was stimulated by a report by Niazi and colleagues in 1981. Specimens of villi were initially obtained from first trimester pregnancies by curettage but in 3 cases, the specimens were obtained by blind transcervical aspiration using a plastic Medicut intravenous cannula attached to a 20 ml syringe. Of these 3 samples, all were successfully cultured and karyotyped using a novel trypsinisation method while the origin of the cells obtained was confirmed by examination of

fetal tissue. In a further paper published in 1981, the same workers reported on the successful gene analysis of chorionic villi as a possible technique for first trimester diagnosis of haemoglobinopathies. Chorionic villi were obtained from the five women studied by blind transcervical aspiration using a Medicut cannula.

A refinement of the blind aspiration method was the guidance of the sampling cannula to the richest source of villi using ultrasound guidance. This idea, first suggested by Niazi and colleagues in 1981, was used in a paper published by Ward and others in 1983, when samples of chorionic villi were successfully obtained in the first trimester by passing a cannula through the cervix and into the placental site using real time ultrasound guidance. All patients were in the first trimester and anaesthetised prior to termination of pregnancy. Their study was divided into three phases. In phase 1, 26 patients between 7 and 12 weeks gestation had sampling performed using a 16 gauge Medicut cannula or a specially designed Portex cannula with one or more side holes. Trophoblast was obtained from 8 patients (31%). In phase 2, the Portex cannula had an end hole in the outer plastic sleeve and an inner aluminium obturator which could be curved appropriately and was visible on the ultra-sound scan. A 20 ml syringe provided greater suction and the presence of villi was confirmed histologically. In 19 patients, between 7 and 13 weeks gestation, trophoblast was obtained in 17 (89%). In phase 3, the same Portex cannula was used as in phase 2, but the samples were examined under a dissecting microscope in the operating theatre, in order

to give instant feedback on the results. Trophoblast was obtained in 19 of 21 patients (90%).

The excellent results of the blind transcervical aspiration reported by the Chinese was encouraging but there were certain problems with their report. They did not indicate whether the cannula was bent to accomodate the angle of the utero cervical canal but they did state that in some cases a tenaculum was applied to the cervix if the uterus was ante or retro flexed. There was no reference in their paper to the number of cases in which aspiration was attempted but villi was not recovered, so the success and problems they had encountered prior to their successful series were not mentioned. The exact materials used in the construction of the cannula were not detailed except that the outer tube was metal. Rhine and Milunsky's report of endocervical aspiration was examined in 1980 by Goldberg and others. Using the same design of Antenatal Cell Extractor (ACE), they were unable to demonstrate that any of the cells they obtained from the 30 patients that they studied in the first trimester, were fetal in origin. As an example, they cited the successful culturing of male fetal tissue from the abortus specimens in 12 patients. In 9 of these cases, the ACE specimens had all been female. Their conclusion was that their inability to demonstrate a male karyotype among the cells retrieved with the ACE cast serious doubts on its usefulness and Rhine and Milunsky's previous results. They attributed their poor results to maternal cell contamination which they stated would be a major problem in the aspiration of trophoblast.

In the paper by Ward describing the Portex cannula, the method of sampling was described but the number of insertions necessary to obtain adequate villi varied from 1 to 5 times, and there was no mention of the number of insertions used in each of the three phases. Similarly, the position of the placental site in the 66 patients reported was 'usually found in the posterior uterine wall' and this may well have influenced their successful results. In phase 2 of their study, when the dissecting microscope was used in the operating theatre, they did not state how this changed their sampling when prior knowledge of the success of the sampling was given to the sampler.

An attempt at comparing the various methods of transcervical chorionic villus sampling was made by Simoni and others in 1983. They tested four different sampling methods for obtaining chorionic villi. Three hundred and seventy-two women at 6 - 12 weeks gestation who were undergoing termination of pregnancy were sampled. Table 2 shows the results of their study. They concluded that the Portex cannula under ultrasound vision was the method of choice. Exact details on the number of insertions used for each cannula was not given but the procedure was repeated not more than three times. Direct feedback on how successful each sampling was, was given at the time of each sampling but no details as to what effect such information had on subsequent attempts at sampling and on any change of direction of the cannula to another position within the uterus, was given. Their technique of transcervical chorionic villus sampling also differed from that reported by Ward. For each case a

TABLE 2

RESULTS OF FOUR DIFFERENT SAMPLING METHODS - SIMONI ET AL 1983

		Patients sampled	Trophoblast obtained
Series I	Hysterscope and Biopsy Forceps Direct Vision	62	47 (76%)
Series II	Abbocath-T 14G Blind Aspiration	159	102 (64%)
Series III	Portex Catheter Blind Aspiration	48	32 (66%)
Series IV	Portex Catheter Ultrasound Guidance	103	99 (96%)

metal uterine sound was first passed through the endocervix to locate its tip on ultrasound and assess the direction of the canal and the length necessary before the placenta was entered. This having been done, the Portex cannula was then guided through a pre-determined path into the proposed sampling site.

The papers of Ward and Simoni popularised the method of transcervical chorionic villus aspiration using the Portex cannula and real time ultrasound guidance. Although, in essence, the apparatus was exactly like the original instrument described by the Chinese in 1975, the addition of real time ultrasound proved to be the key to the improvement in sampling success.

Having obtained samples of the Portex cannula in 1983, and used it on patients prior to termination of pregnancy, several problems were encountered. The biggest problem related to the fact that the outer plastic sleeve of the cannula which retained its shape as long as the inner aluminium obturator was in place, did not retain its shape when the aluminium obturator was withdrawn. As the instrument usually had to be bent to the appropriate angle to negotiate the utero cervical canal, withdrawing the bent aluminium obturator moved the more rigid plastic cannula so that it then attempted to return to its original shape. The effect of this was that the cannula tip changed position and its ultrasonic image was 'lost'. The ability to introduce the Portex cannula into uterine positions that demanded a more acute bend on the cannula proved difficult.

In the 1983 paper by Simoni et al, the placental position defined on the ultrasound image was not given for any patient. There were no details on any difficulties in introducing the Portex cannula into different placental positions. In Ward's paper, the placental position was 'usually' found on the posterior uterine wall. Thus the introduction of the cannula to this site may have been easier than to anterior wall placental sites or placental sites near the fundus of the uterus. The fact that the placenta was usually placed posteriorly in their study may have favourably biased their results as the degree of bend on the Portex cannula may have been kept to a minimum and hence reduced the distortion of the image of the plastic cannula as the inner metal obturator was withdrawn. In a more inaccessible placental site, where the degree of bend on the Portex cannula would be greater, the risk of significant shift of the plastic cannula and loss of its ultrasound image could have been greater and led to less successful chorionic villus recovery.

4.2 PROPOSED STUDIES

With the criticisms of Ward and Simoni's studies in mind, two metal cannulae were designed and constructed. Familiarity with the technique of ultrasonically guided transcervical chorionic villus aspiration was gained in a pilot study involving 88 aspirations in 32 patients in the first trimester (Table 3). The pilot study enabled experience with ultrasound guided transcervical chorionic villus sampling to be obtained while eliminating operator bias when using the different cannulae. Thereafter, a randomised study was

TABLE 3

RESULTS OF THE PILOT STUDY

Cannula	No. of patients	Total no. of insertions	Villi obtained
Portex (P)	29	46	19 (41.3%)
Malleable Stainless Steel (MSS)	20	26	16 (61.5%)
Aluminium (AL)	9	16	6 (37.5%)

performed that compared the Portex cannula with the two personally designed metal cannulae. Other factors that might be expected to effect successful villus recovery were also examined.

4.3 DESIGN OF CANNULAE

The Malleable Stainless Steel Cannula (MSS) and the Aluminium Cannula (AL) were compared with the Portex Trophocan (P) in a random study with special reference to villus recovery rates and subsequent karyotyping as it is in the field of chromosomal diagnosis that chorionic villus sampling is likely to find its widest application.

Two cannulae were designed and constructed (with the assistance of Rocket of London Limited) to fit six criteria.

(1) The cannula should have a large enough bore that villi could be aspirated, but small enough overall diameter to negotiate the endocervical canal, without the need for prior dilatation.

(2) The cannula should be easily seen on the ultrasound image especially its tip.

(3) The cannula should have a 'memory', that is, it should retain its shape when bent but the process of bending should not narrow the lumen of the cannula.

(4) The material used in the cannula should not be toxic to the fetus or mother.

(5) The cannula should be able to withstand sterilisation procedures.

(6) The cannula should be made to fit standard syringe fittings.

A) Malleable Stainless Steel Cannula (MSS)

(Figure 2)

The features of this cannula fulfil all six of the criteria, but particular emphasis was placed on the ability of the tip to be seen on the ultrasound image. The way this was done can be seen on Figure 2. The tip was made bulbous but streamlined, to have an overall olive shape and contained two opposing holes through which the villi could be sucked. This design was chosen with the idea that no matter what position the tip was within the villi, a hole would always be facing the villi at an approximate 90° angle.

B) Aluminium Cannula (AL)

(Figure 3)

All six criteria were fulfilled by this cannula. The tip was rounded to avoid trauma at insertion. It also had the two opposing holes design used in the Malleable Stainless Steel cannula at the tip. The cannula was of uniform diameter throughout its length. The characteristics of the two metal cannulae and the Portex cannula are summarised in Table 4.

4.4 SUCTION PRESSURE STUDIES

Each of the cannulae used in the pilot and randomised studies was assessed as to the negative pressures developed along them when 5 or 10 mls of 'suction' was applied using the syringe. No details had been given in any previous study of the suction pressure developed along the Portex cannula. I felt that large differences in suction pressure between the cannulae would bias the results, and therefore this area

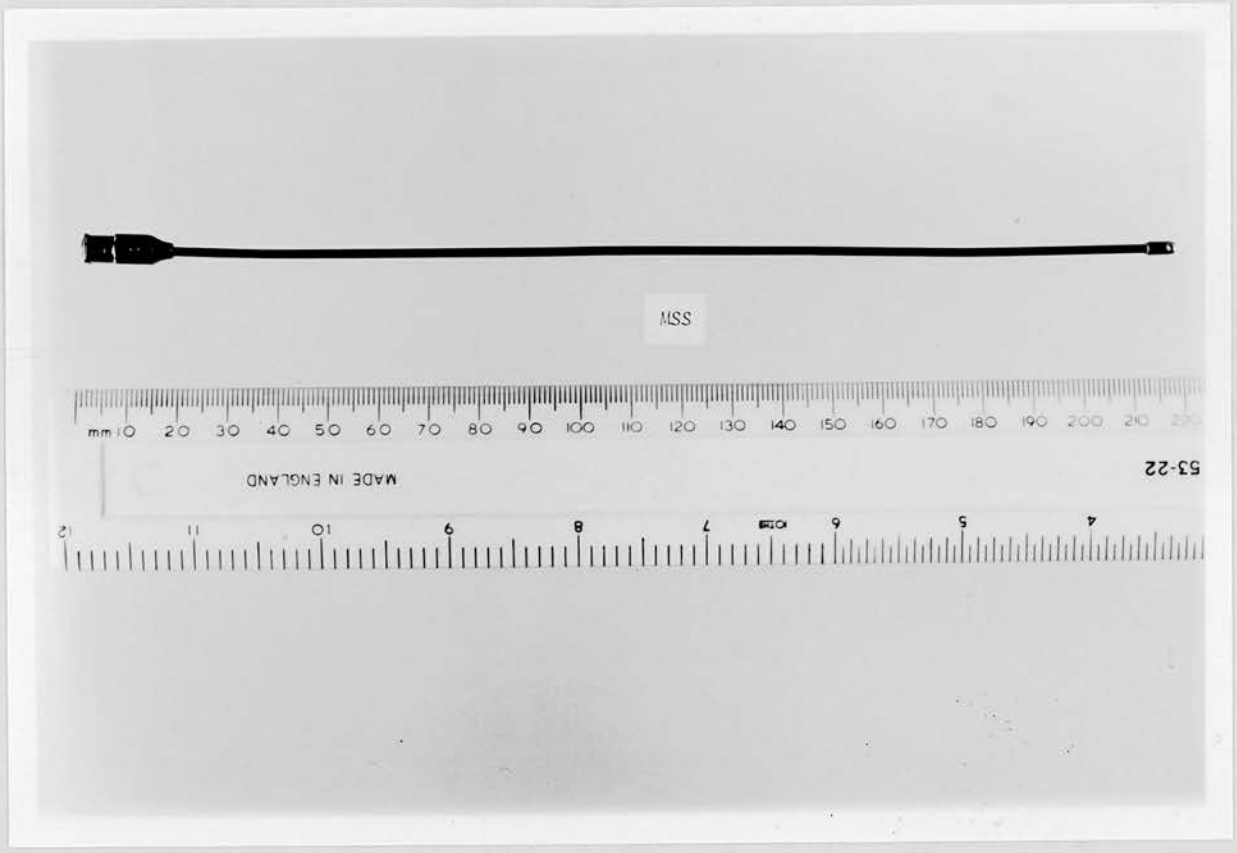


FIGURE 2: MALLEABLE STAINLESS STEEL CANNULA

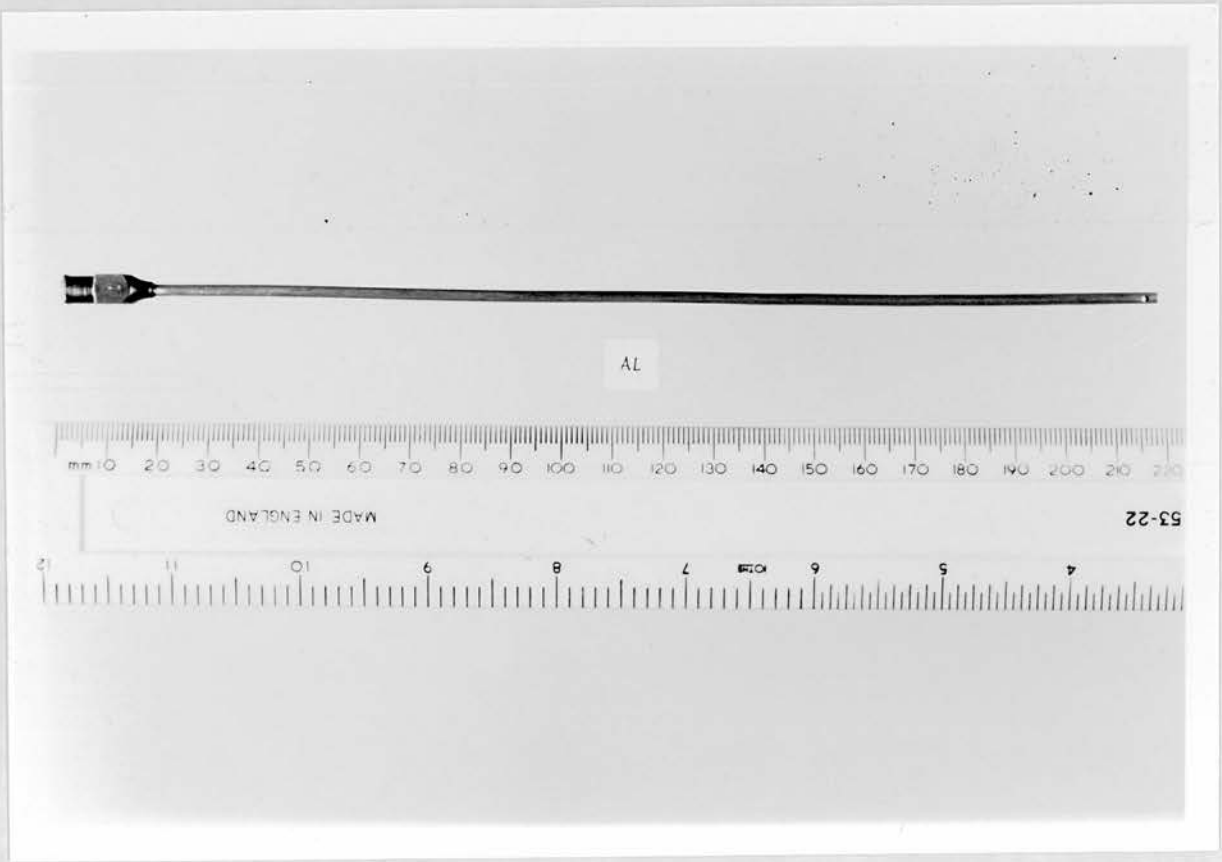


FIGURE 3: ALUMINIUM CANNULA

TABLE 4

CHARACTERISTICS OF CANNULAE

Cannula	Length mm	Internal Diameter mm	Outer Diameter mm
Portex Trophocan (P)	210	1.13	1.45
Malleable Stainless Steel (MSS)	210	1.80*	2.70
Aluminium (AL)	210	1.30	2.30

* at olive tip

needed investigation. The measurement of vacuum developed along the cannulae was performed using a monolithic pressure transducer, the SENSYM LX0503A.

A diagram of the apparatus used is given in Figure 4. With the end of the cannula blocked by placing it into a placenta, the syringe was withdrawn to 5 mls and 10 mls, five times for each cannula and the pressure read directly from the Digital Volt Meter attached to the pressure transducer.

The results seen in Table 5 for all cannulae, show the negative pressures developed along the cannulae. At 5 mls of syringe withdrawal, the negative pressure is highest for the Portex cannula and lowest for the Aluminium cannula. At 10 mls, the Aluminium is the lowest and there is no difference between the Portex and the Malleable Stainless Steel cannulae.

4.5 RANDOMISED CANNULA STUDY

A) Patients

Fifty patients between 8 and 12 weeks gestation, as assessed on ultrasound measurement (mean 9.4 ± 0.7 weeks) were enrolled into the study. All were undergoing termination of pregnancy for non genetic reasons. Informed consent was obtained in every case. Local ethical committee approval was obtained. Only viable pregnancies as demonstrated by ultrasound were studied. One twin pregnancy was excluded from the study. Sampling was performed in the 24 hours prior to termination.

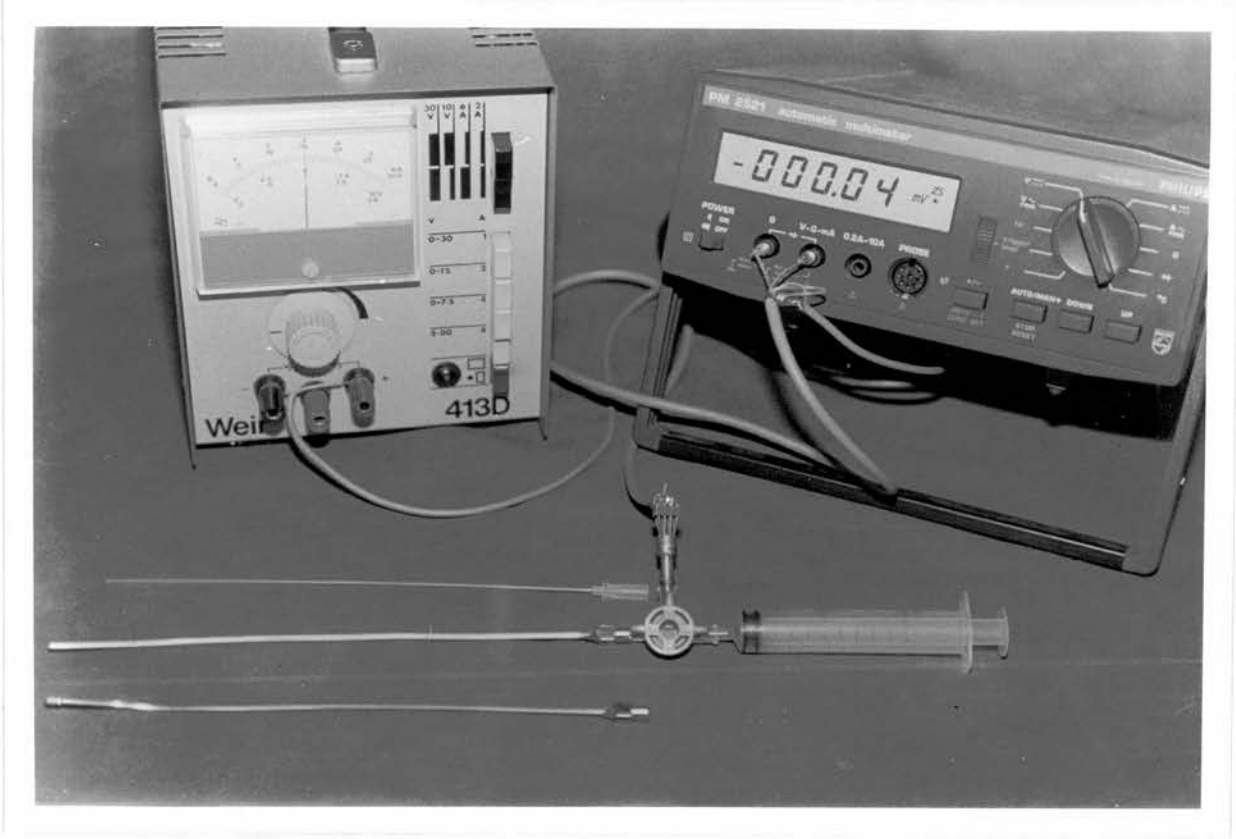


FIGURE 4: TEST RIG FOR SUCTION PRESSURE EXPERIMENT. CANNULA CONNECTED VIA 3 WAY TAP TO SYRINGE AND PRESSURE TRANSDUCER. PRESSURE TRANSDUCER CONNECTED TO DIGITAL VOLTMETER (POWER SUPPLY ON LEFT)

TABLE 5

RESULTS OF PRESSURE STUDIES

		Pressure (mm.Hg.)					Mean	S.D.	*Dead space volume (ml)
		1	2	3	4	5			
P cannula syringe withdrawal	5 ml	-625	-626	-627	-625	-624	-625.0	0.25	0.6
	10 ml	-672	-672	-671	-671	-671	-671.4	0.23	
MSS cannula syringe withdrawal	5 ml	-613	-612	-614	-614	-615	-613.6	0.32	0.7
	10 ml	-672	-672	-671	-671	-671	-671.4	0.23	
AL cannula syringe withdrawal	5 ml	-597	-597	-598	-598	-597	-597.4	0.23	0.8
	10 ml	-660	-659	-660	-659	-659	-659.4	0.23	

P Portex
MSS Malleable Stainless Steel
AL Aluminium
S.D. Standard Deviation

* The dead space volume is the volume of the 3 way tap, the cannula and the transducer cavity.

B) Sampling Method

No anaesthesia or analgesia was used before or during sampling. A single operator technique was used. With the ultrasound transducer in the left hand, the cannula was guided to the sampling site, as identified on the ultrasound image. Preliminary ultrasonography (using the ADR 4000 Sector Scanner, with a 3 MHz Probe) was carried out to verify gestational age of the fetus by crown rump length measurements, and to confirm viability. The placental site was visualised and assigned to a predominantly anterior or posterior uterine position.

A sterile Cusco's speculum was passed into the vagina and the cervix visualised. The position and angle of the utero cervical canal was assessed and the cannula bent to the same angle. Using continuous real time ultrasound guidance, the cannula was passed through the cervix and the image of the cannula tip observed. Under ultrasound guidance, the cannula tip was manoeuvered to the placental site which was entered to a depth of approximately 1 cm, to overcome the problem of sampling degenerating villi at the placental edge. Ten millilitres of suction was applied and with a slight hoovering action and with suction maintained, the cannula was withdrawn. Each sample was collected into 5 mls of F10 medium containing 20% Fetal Calf Serum, for subsequent cytogenetic analysis. Each cannula was passed twice and this was designated a "procedure". Ward had shown that two passages of the Portex cannula were associated with an 80% villus recovery rate and therefore it was considered that this would be sufficient for this study.

C) Random Allocation of Cannula

Prior to the commencement of the study, one of the three types of cannulae was selected at random and assigned to a procedure. This random allocation was repeated for 100 procedures. Each patient had two procedures carried out. The first patient was therefore assigned the first two cannulae and for the second patient the next two cannulae and so on (Appendix 1). Thus, in the majority of patients, two different cannulae types were used but in a proportion of patients (38%), the random allocation resulted in two cannulae of the same type being used on a single patient, Portex (3/50), Malleable Stainless Steel (8/50) and Aluminium (8/50). The random allocation of a cannula type to each procedure resulted in the Portex cannulae being used in 15 first procedures and 17 second procedures, the Malleable Stainless Steel being used in 20 first procedures and 17 second procedures and the Aluminium being used in 15 first procedures and 16 second procedures. The variation in the distribution of cannula types between the first and second procedures is not significant ($F = 1.47$ $p > 0.05$).

After sampling each patient had ultrasonography performed and the viability of the pregnancy and state of the sampling site were noted.

D) Cytogenetic Analysis

No direct feedback on success or otherwise of the first sample was given before the second sample, (constituting the procedure) was taken. The villus samples were sent to one of two cytogenetic laboratories and the weight of the villi

obtained for each sample was assessed by the Cytogeneticist with reference to samples of known wet weight. Three weights were recorded, < 5 mg, 5 - 10 mg, > 10 mg.

The villi were treated by the overnight culture method described by Simoni et al or by a direct method as described by Burgoyne (Burgoyne 1983). Improved chromosome morphology was obtained by fixing the villi at least overnight. Standard G-banding techniques were employed. The cytogenetic sampling was deemed to be adequate if a karyotype was obtained from a minimum of 2 banded mitoses.

E) Data Recorded

The following data was recorded by the operator (Appendix 2 - sample of data sheet used) parity, gestation by dates, gestation by ultrasound estimation and placental position. Patient reaction to the procedure was graded arbitrarily by the operator from 0 to 3. Nought was no discomfort recorded and 3 was pain such that the procedure was abandoned. Difficulty in passing the cannula through the cervix was recorded on a scale 0 to 3, with 0 being no difficulty and 3 being the added use of a volsellum applied to one lip of the cervix to give counter traction to the forward movement of the cannula. Bleeding was graded as none, spotting and fresh bleeding. This referred to bleeding during or immediately after sampling. Visualisation of the cannula (by the operator) on the ultrasound screen during the procedure was recorded as good, poor and not seen. In practice however, in no case was the cannula not seen.

4.6 RESULTS

Table 6 shows the age, gravidity and gestational age as calculated from crown rump length measurement and indicates that there is no difference in the groups of patients assigned to each cannula when these criteria are considered. Each patient is represented twice, as two procedures were carried out per patient. The success of a second procedure may have been influenced by the outcome of the first procedure. However, on examination, the success in obtaining villi in a first or second procedure, irrespective of the cannula used, was not significantly different (Table 7).

Table 8 and Figure 5 show the villus recovery rate and the proportion of procedures where karyotypes were obtained for the three cannulae. Villus recovery (all weights) was greatest with the Stainless Steel cannula so that with two passes of a cannula, villi were obtained approximately 68% of the time. However, the differences between the three cannulae in successful villus recovery was not significantly different at the 95% level. When the proportion of karyotypes obtained is considered, the Aluminium cannula appears to be most effective and the Portex cannula the least, and this difference is significant ($0.05 > p > 0.01$; Figure 5). It has been reported previously that 10 mgs of villus material is required for reliable karyotyping and this is confirmed by the present study for each of the three cannulae (Table 9). When the quantity of villi obtained by each cannula per procedure is examined, it can be seen that the metal cannulae are comparable and obtain > 10 mgs of tissue in 27 - 35% of procedures. The Portex cannula in contrast

TABLE 6

PATIENT AGE, GRAVIDITY AND GESTATION

	Cannulae		
	P	MSS	AL
Age Distribution (years)			
Mean	24.0	25.0	24.2
S.D.	5.4	6.2	5.7
Parity			
Mean	1.4	1.1	1.4
S.D.	1.2	1.0	1.2
Gestation (weeks)			
Mean	9.1	9.3	9.7
S.D.	0.7	0.9	0.9

P Portex Trophocan
MSS Malleable Stainless Steel
AL Aluminium

S.D. Standard Deviation

TABLE 7

CHORIONIC VILLUS SAMPLING SUCCESS PER PROCEDURE

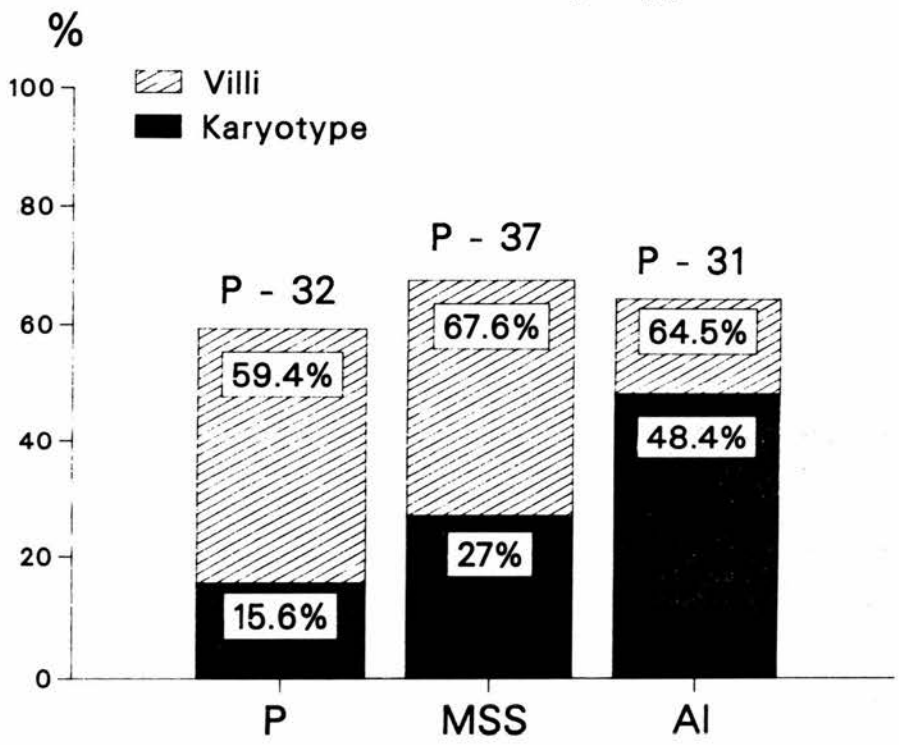
	1st procedure		2nd procedure		X^2	P
	No.	%	No.	%		
Success in obtaining villi	30	60	34	68	0.7	0.5>p>0.25

TABLE 8

VILLI AND KARYOTYPE BY CANNULA

Cannula	Villi obtained		Karyotype obtained		Total number of procedures
	N	%	N	%	
P	19	59.38	5	15.63	32
MSS	25	67.57	10	27.03	37
AL	20	64.52	15	48.39	31
Total	64	64.00	30	30.00	100

N Number of procedures
P Portex
MSS Malleable Stainless Steel
AL Aluminium



P - Procedures

1 Procedure = 2 Sampling Attempts

	Chi Squared	p
Villus Recovery	0.5	p>0.05
Karyotype Success	8.3	0.05>p>0.01

FIGURE 5

TABLE 9

KARYOTYPE SUCCESS FOR DIFFERENT QUANTITIES OF VILLI OBTAINED PER PROCEDURE

	Quantity of villi (mg)					
	<5		5-10		>10	
Cannula	N	%	N	%	N	%
P	10	10.0	5	0.0	4	100.0
MSS	12	33.3	3	33.3	10	50.0
AL	4	25.0	5	100.0	11	81.8

N Number of procedures where villi obtained
% Karyotype success
P Portex
MSS Malleable Stainless Steel
AL Aluminium

only obtains > 10 mgs of villi in 12.5% of procedures (Table 10). Two main factors that may be expected to influence chorionic villus sampling are placental site and parity. The difference in villus recovery by placental site is not significant, but there is a 16% difference in the mean villus recovery rate depending on the sampling site. This is shown in Tables 11 and 12 , and diagrammatically in Figure 6. Although a poorer recovery rate of villi was expected and hence reduced karyotype success in nulliparous patients, because of anticipated difficulty negotiating the endocervical canal, no such difference was observed (Table 13).

The ease of insertion of the cannulae, where no difficulty was encountered (score '0'), is seen in Table 14. There was a significant difference in the ease with which the metal cannulae and especially the Aluminium cannula could be inserted compared with the Portex cannula. In 11 procedures, the added use of the tenaculum was needed and it is interesting to note that all of these related to nulliparous patients only (Table 15).

The ability to continuously visualise the cannula tip on the ultrasound screen was recorded for each cannula by the operator (Table 16). Visualisation of the Malleable Stainless Steel cannula was best overall, with good visualisation being recorded in 86% of procedures, compared with 72% with Portex, reflecting the enhanced ultrasound image of the olive tip, but the differences statistically between the three cannulae were not significant.

Pain and bleeding during cannula insertion are recorded in Tables 17 and 18, and although there was a significant

TABLE 10

VILLUS RECOVERY BY WEIGHT

Weight of villi (mg)	No villi		Procedures where villi obtained						Total Number of procedures
			<5		5-10		>10		
	N	%	N	%	N	%	N	%	
P	13	40.6	10	31.5	5	15.6	4	12.5	32
MSS	12	32.4	12	32.4	3	8.1	10	27.0	37
AL	11	35.5	4	12.9	5	16.1	11	35.5	31
Total	36	36.0	26	26.0	13	13.0	25	25.0	100

N Number
P Portex
MSS Malleable Stainless Steel
AL Aluminium

TABLE 11

VILLI BY PLACENTAL SITE

Cannula	POSTERIOR			ANTERIOR		
	Villi obtained	Total no. of procedures	%	Villi obtained	Total no. of procedures	%
P	15	22	68.18	4	10	40.00
MSS	17	23	73.91	8	14	57.14
AL	13	19	68.42	7	12	58.33
Total	45	64	70.31	19	36	52.78

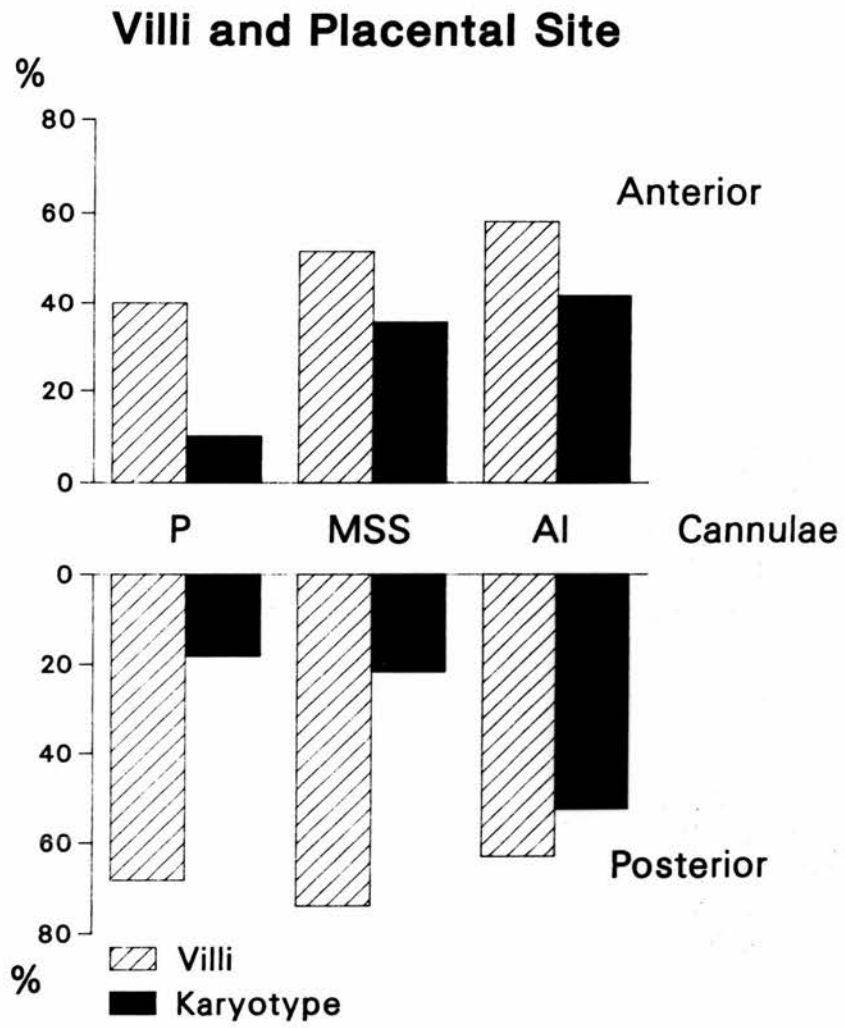
P Portex
MSS Malleable Stainless Steel
AL Aluminium

TABLE 12

KARYOTYPE BY PLACENTAL SITE

	POSTERIOR			ANTERIOR		
Cannula	Karyotype obtained	Total no. of procedures	%	Karyotype obtained	Total no. of procedures	%
P	4	22	18.18	1	10	10.00
MSS	5	23	21.74	5	14	35.71
AL	10	19	52.63	5	12	41.67
Total	19	64	26.69	11	36	30.56

P Portex
MSS Malleable Stainless Steel
AL Aluminium



	F	P
Villi Obtained	6.2	>0.05
Karyotype Success	0.7	>0.05

FIGURE 6

TABLE 13

PARITY AND CHORIONIC VILLUS SAMPLING SUCCESS

Number of previous births		0	1	2	3	4
Number of patients		16	11	16	4	3
Number of procedures		32	22	32	8	6
Villi obtained	N	21	16	19	6	3
	%	65.6	72.7	59.3	75	50

N Number

TABLE 14

EASE OF INSERTION OF CANNULA INTO THE CERVIX

Cannula	Score '0'		Total number of procedures
	N	%	
P	16	50.0	32
MSS	27	73.0	37
AL	26	83.0	31
X^2		8.92	
p		0.05>p>0.01	

N Number
P Portex
MSS Malleable Stainless Steel
AL Aluminium

TABLE 15

EASE OF INSERTION OF CANNULA INTO THE CERVIX

Cannula	Score '3'		Total number of procedures
	N	%	
P	7	21.9	32
MSS	2	5.4	37
AL	2	6.5	31

N Number
P Portex
MSS Malleable Stainless Steel
AL Aluminium

TABLE 16

VISUALISATION OF CANNULA

Cannula	Good visualisation N	Good visualisation %	Poor visualisation N	Poor visualisation %	Total number of procedures
P	23	71.9	9	28.1	32
MSS	32	86.5	5	13.5	37
AL	23*	74.2	7	22.6	30
X^2			1.68		
p			p>0.05		

N Number
P Portex
MSS Malleable Stainless Steel
AL Aluminium

* 1 procedure had differing values for each insertion of the same cannula therefore was omitted.

TABLE 17

PAIN DURING CANNULA INSERTION

Cannula	Score '0'		Score 0		Total number of procedures
	N	%	N	%	
P	20	62.5	12	37.5	32
MSS	30	81.1	7	18.9	37
AL	29	93.5	2	6.5	31
X^2	10.19				
p	$0.001<p<0.01$				

N Number
P Portex
MSS Malleable Stainless Steel
AL Aluminium

TABLE 18

BLEEDING DURING CANNULA INSERTION

Cannula	Score '0'		Score not '0'		Total number of procedures
	N	%	N	%	
P	28	90.3	3	9.7	31*
MSS	30	81.08	7	18.92	37
AL	26	86.6	4	13.4	30**
X^2			1.6		
p			p>0.05		

N Number
P Portex
MSS Malleable Stainless Steel
AL Aluminium

* Uncertain score for 1 patient

** No score recorded for 1 patient

difference in discomfort with the Portex cannula compared with the metal cannulae, there was no significant difference in the bloodless sampling rates for all procedures.

The Aluminium cannula proved to be best overall in villus recovery and subsequent karyotyping. This could be a reflection of the negative pressure developed along the cannula when 10 mls of suction were applied. However, the vacuum pressure studies have shown that the Portex and Malleable Stainless Steel cannulae have a higher negative pressure than the Aluminium cannula and this may lead to fragmentation and destruction of villi more often than with the Aluminium cannula.

4.7 DISCUSSION

This study compared the Portex cannula with the two malleable metal cannulae and the results indicate the superiority of the metal cannulae with respect to villus recovery and the subsequent preparation of chromosomes from the samples obtained. The technique of transcervical vacuum aspiration used, relied solely on the successful guidance of the cannula into the placental site using real time ultrasound imaging.

Thus the study examines the inherent qualities of each cannula studies, matching the method of sampling with that reported by Ward and Brambati. The randomised study does not mimic exactly the diagnostic situation where instant feedback on the success or otherwise of each aspiration can influence the direction and depth of cannula insertion. From the results of the randomised study, the preferred instrument for sampling would be the Aluminium or Malleable Stainless Steel cannula.

The results have shown that a cannula could be passed through the cervix into the uterus without pain, in up to 93% of patients, of whom 30% were nulliparous. None of the 50 pregnancies studied had a significant bleeding episode up to the termination and in no case were the membranes ruptured. No miscarriages were caused by the chorionic villus sampling in any of the 50 cases. The Aluminium cannula produced the best karyotype results reflecting the better villus quantity per sample. The efficacy of any instrument used to obtain chorionic villi should be judged on the frequency with which 10 mgs or more of villi are obtained for a given number of passages of an instrument.

Although many authors report villus recovery rates of up to 90% using transcervical aspiration, they do not usually quote the karyotype success per passage of cannula which does not reflect the overall villus recovery rate for amounts less than 10 mgs. The number of insertions of the instrument required before adequate amounts of villi are obtained, vary from once to five times in reported series (Brambati et al 1985; Ward et al 1983; Warren et al 1985). In the method described and within the limitations already set in this study, villus recovery rates were no better than 74% when a cannula was passed twice and the placenta was posteriorly sited. In those patients, who after randomisation happened to draw the same cannula for both procedures (4 samples), the recovery rate remained constant at 89% for all cannulae but only when a posterior placental site was sampled. This may explain Ward et al's results, as in their series the sampling site was 'usually posterior'.

Reported series do not state the sampling site when reporting on their ability to recover chorionic villi using a particular instrument (Brambati et al 1985 a; Ward et al 1983), but this study has shown that with anteriorly sited placentae, the karyotype success rate is reduced compared to that from posteriorly sited placentae and this effect was most pronounced for the Portex cannula. The explanation for this could be that the loss of ante flexion of the plastic Portex cannula after removal of the aluminium obturator causes the tip of the cannula to move away from the villi and towards the membranes. It may also be that the positive location of the placenta by ultrasound was incorrect more often when the placenta was situated anteriorly than posteriorly.

The correct assignment of placental position by ultrasound is the key to successful chorionic villus sampling. Positive proof of the accuracy of such an ultrasound assignment can only be given by recovering villi in diagnostic amounts after the cannula is seen to enter the specified area within the uterus. Although the placental site has certain ultrasonically identifiable characteristics, mistakes in its localisation could occur. Similarly, the results of any series reporting on successful villus recovery may be biased if the bulk of the placentae were in the more favourable posterior position. In this study, the placenta was assigned to a mainly posterior position in 32 (64%) and to a mainly anterior position in 18 (36%).

When guiding any biopsy instrument under real time grey scale ultrasound guidance through tissue layers, continuous positive visualisation of the tip of the instrument can be

difficult. The tip of the instrument itself may not be echogenic enough to distinguish it from the echoes produced by the tissue layers. This may be improved by enhancing the echogenicity of the tip of the instrument either by making it physically large (as in the case of the Malleable Stainless Steel tip) or enhancing the echoes from the instrument as a whole and electronically subtracting the echoes from most of the length of the instrument except the tip (McDicken et al 1984).

These studies indicated that other methods than transcervical sampling may be necessary to obtain villi when the placental position was anteriorly placed. Before the application of transcervical chorionic villus sampling diagnostically, a study into transabdominal chorionic villus sampling was undertaken examining the feasibility of the technique and the effect of placental site on sample weight as well as comparing transabdominal and transcervical chorionic villus suction aspiration.

TRANSABDOMINAL CHORIONIC VILLUS SAMPLING USING SUCTION ASPIRATION

5.1 INTRODUCTION

In a paper discussing future trends in the prenatal diagnosis of fetal chromosomal disorders, Warburton and Miller (1972) quoted the transabdominal placental biopsy technique of Alvarez (1966), as a technique that could be suitable for use in detecting such disorders. Alvarez (1966) had published details of 3 cases where the diagnosis of hydatidiform mole was made by inserting a needle through the abdominal wall into the pregnant uterus and aspirating molar vesicles. He had used the same technique in a further 50 pregnancies from 10 to 40 weeks gestation, although no details of the outcome of these pregnancies was reported.

Aladjem (1969) had used placental biopsies obtained by transabdominal needle aspiration to investigate pathological changes in the placenta of pregnancies complicated by diabetes mellitus, pre-eclampsia and prolonged pregnancy. A total of 215 third trimester transabdominal samples were obtained with no established fetal or maternal complications. These procedures were performed before the advent of real time ultrasound. Aladjem stated that the limitations of the technique was in the case of posterior implantation of the placenta. It was not until the publication in 1984 of a paper by Smidt-Jensen and Hahnemann that transabdominal chorionic villus sampling in the first trimester was used as a diagnostic method for fetal genetic diagnosis.

Smidt-Jensen's paper reported details of 58 women who had transabdominal chorionic villus sampling attempted prior to termination of pregnancy. The average gestational age was 9 weeks. In 95 transabdominal punctures, villi were successfully obtained in 78 (82%). Samples were obtained under real time ultrasound guidance. A needle guide was attached to the ultrasound transducer and the direction of the needle was predetermined by first identifying the track it took on the ultrasound screen using a water bath for the test. The track was marked on the screen with a marker pen. During transabdominal chorionic villus sampling, the appearance of the uterus on the screen was then adjusted by altering the position of the transducer until the proposed needle track (already drawn on the screen) traversed the placental area as identified within the uterus. An 18 gauge, 150 mm spinal needle was inserted into the uterus (along the predetermined track) and just into the placental edge. The depth and direction being observed on the ultrasound image. Once the placental edge was reached, the trochar of the 18 gauge needle was removed and a 22 gauge, 200 mm spinal needle was introduced down the 18 gauge cannula and into the placental substance. A 20 ml syringe, attached to the 22 gauge needle was used to aspirate villi. The syringe contained a few millilitres of Hanks solution.

No details on the placental position in any of the 58 women tested were given. Details on the villus weights obtained using this method and the subsequent karyotype success were not given. Similarly, the quality of the needle

visualisation and any difficulties in sampling from retroverted uteri were not stated.

In October 1984, I visited Hahnemann's unit in Aalborg, Denmark and the technique described in their paper was demonstrated to me by Dr Stein Smidt-Jensen. Having witnessed the technique, a pilot study was undertaken by me in Birmingham to assess feasibility and to adapt and modify the technique to the equipment which was available.

5.2 PILOT STUDY OF TRANSABDOMINAL CHORIONIC VILLUS SAMPLING

The aims of the pilot study were:-

(1) To design equipment that could be used for transabdominal chorionic villus sampling using available ultrasound equipment.

(2) To test the equipment in a suitable group of patients.

(3) To gain experience in the method of transabdominal chorionic villus sampling.

5.3 MATERIALS

A) Ultrasound

The ultrasound machine used in the transcervical study, the ADR 4000 SL, was used. This machine had a 14 mm end-fire sector transducer (Figure 7). The transducer handle had longitudinal grooves on it that marked the plane of the ultrasound beam, helping the operator to correctly orientate the ultrasound image.

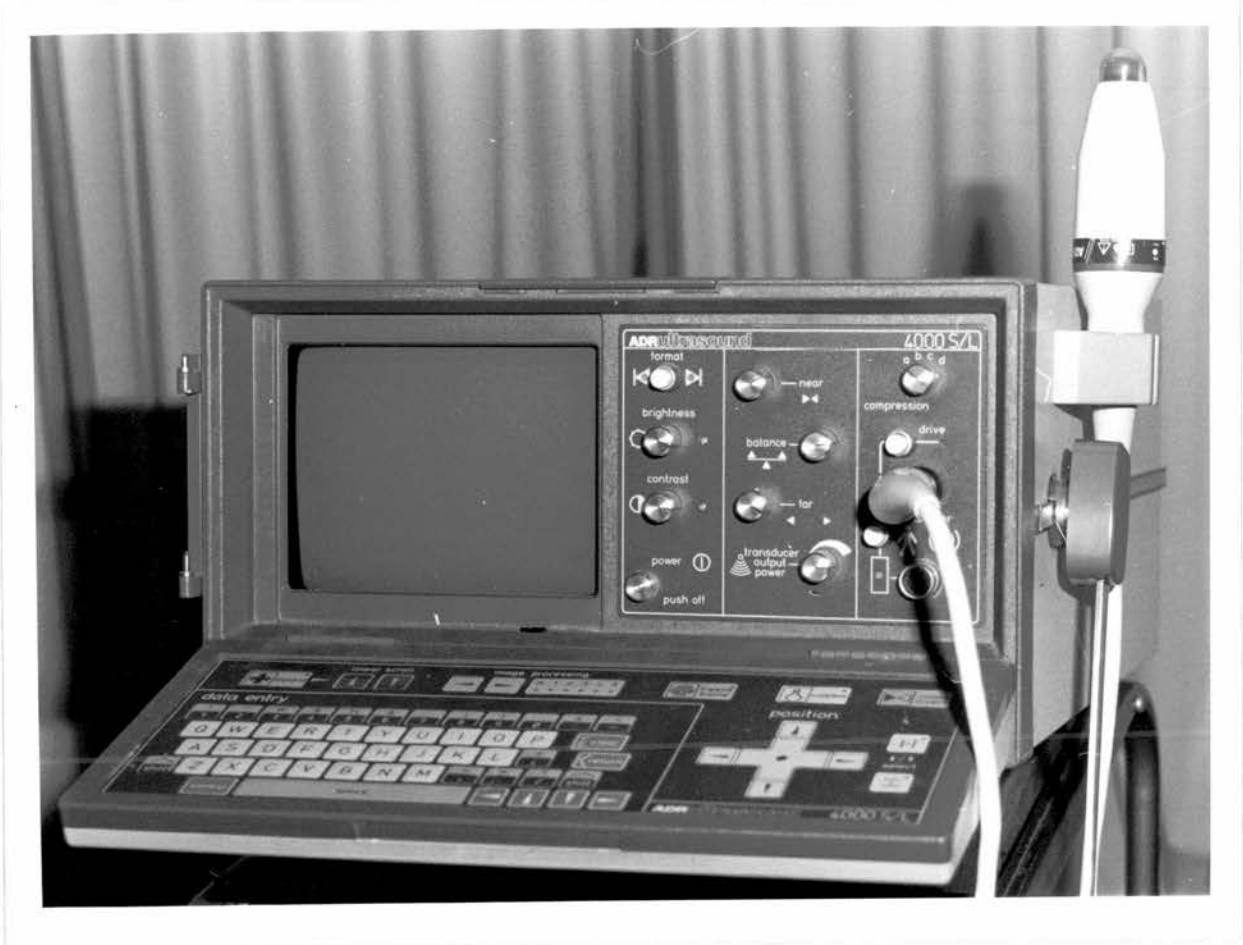


FIGURE 7: THE ADR 4000 S/L WITH 14 MM END FIRE SECTOR TRANSDUCER

B) Design of Equipment

(i) Needle Holder

This device was designed and constructed by Mr G Hollins of the Medical Physics Department, Birmingham Maternity Hospital, to specifications drawn up by myself. Its main features were:-

(1) An aperture into which the ultrasound transducer could fit and be held without moving.

(2) A slot, just wide enough for the placement of an 18 gauge spinal needle (Figure 8).

(3) A spring loaded button which compressed the needle against the floor of the slot but allowed upwards and downwards motion of the needle.

(4) Grooves on the transducer holder allowing accurate matching to the grooves on the transducer so that the ultrasound beam would always be in a constant position to the needle slot (Figure 9).

(5) A material that would not damage the ultrasound transducer yet be sterilisable.

(ii) Guide for the Needle Image

The image of the needle as it passed into the ultrasound beam was displayed on the screen of the ultrasound machine by placing the transducer and needle assembly with the transducer in place just into a water bath.

By correctly aligning the transducer grooves to the grooves on the holder, the track of the needle on the ultrasound image was always on the same place on the screen. This place

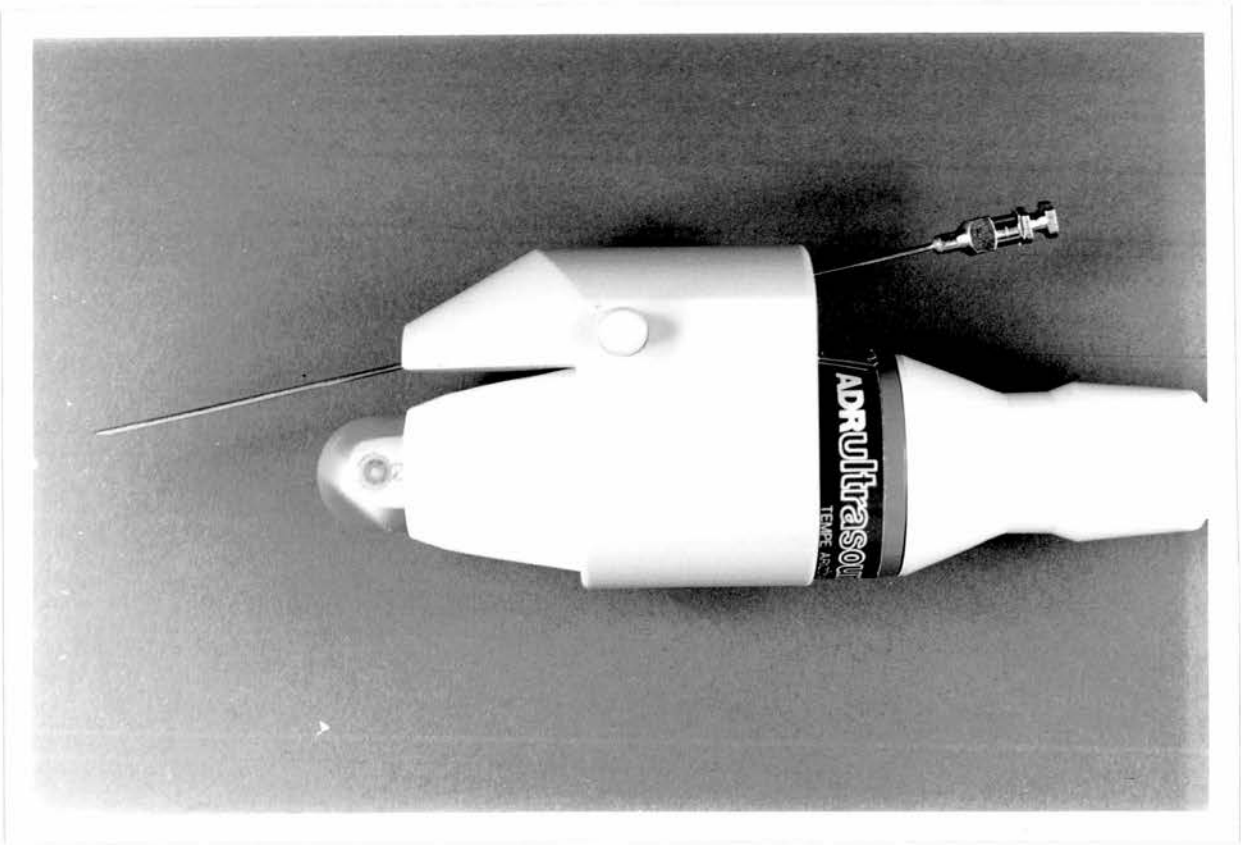


FIGURE 8: THE NEEDLE HOLDER ATTACHED TO THE SECTOR TRANSDUCER WITH THE 18 GAUGE SPINAL NEEDLE IN PLACE

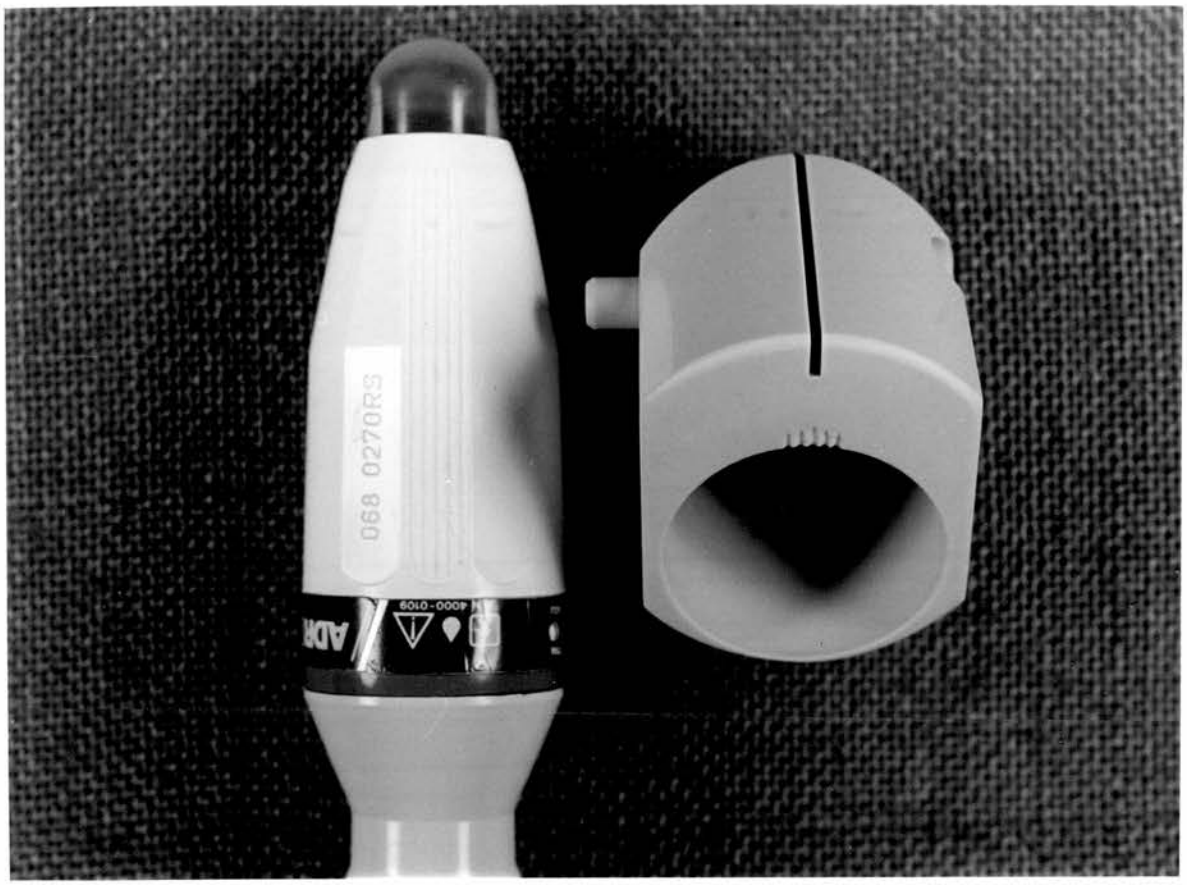


FIGURE 9: TRANSDUCER AND NEEDLE HOLDER SHOWING MATCHING GROOVES

was marked on to a perspex plate placed over the screen of the ultrasound machine (Figures 10 and 11). Thus, prior to each transabdominal sampling, the proposed direction of the needle on the ultrasound image was known by placing the perspex plate over the ultrasound screen.

During the scanning of a pregnant uterus, the image of the placenta obtained was manipulated (by moving the plane of the scan) until the needle track marked on the perspex screen, was seen to intersect with the placenta.

5.4 METHOD OF CHORIONIC VILLUS SAMPLING

Under real time ultrasound guidance and with the needle track marked, as already described, an 18 gauge, 150 mm spinal needle was inserted through the abdomen and into the uterus (via the transducer and needle guide assembly).

The trochar of the 18 gauge needle was removed and the smaller 22 gauge, 200 mm spinal needle to which a 20 ml syringe was attached, was introduced down the 18 gauge cannula and the villi cut and aspirated. The syringe contained a few millilitres of F10 medium.

A) Patients

Thirty four patients with a mean gestational age of 9.5 weeks (range 8 to 12.5, S.D. 1.2) were recruited. Informed consent was obtained in each case. All procedures were carried out under general anaesthesia prior to therapeutic termination of pregnancy.

There were 33 singleton pregnancies and one twin pregnancy.

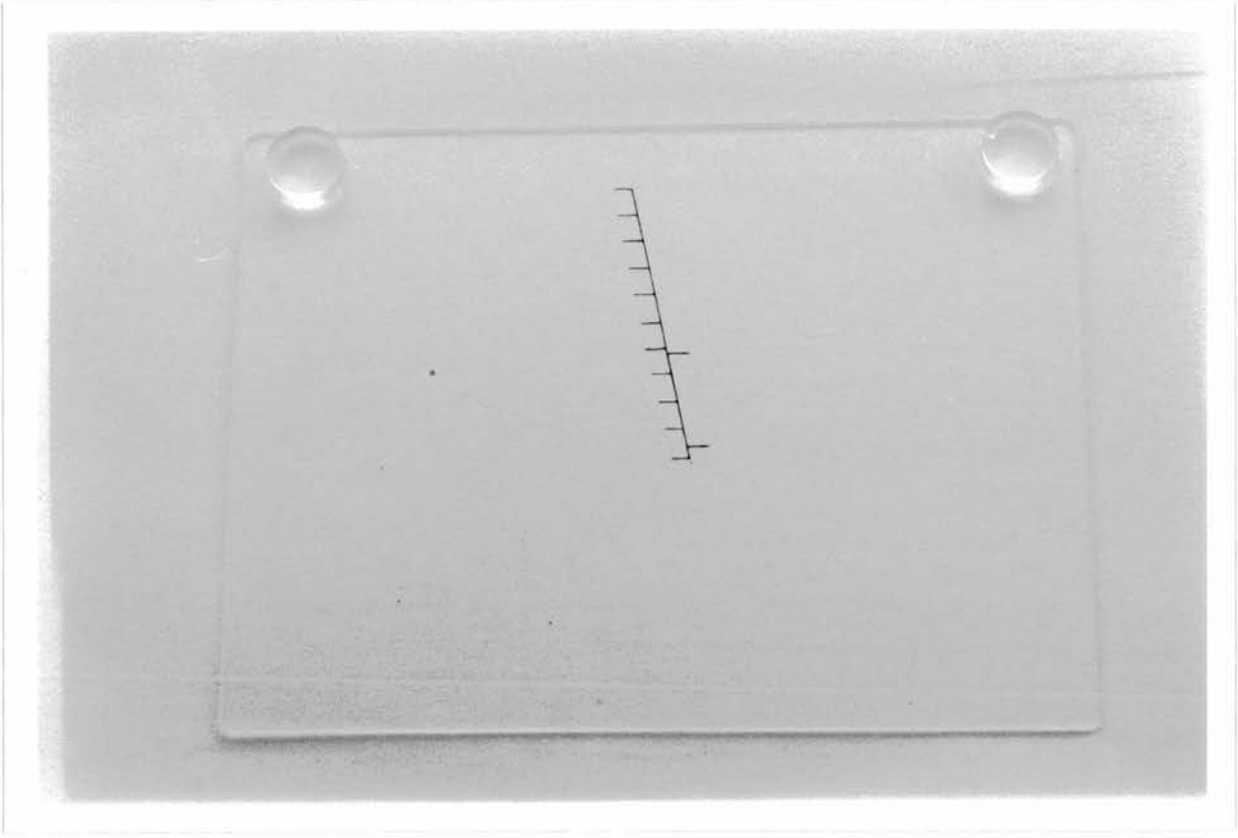


FIGURE 10: PERSPEX PLATE WITH INSCRIBED LINE INDICATING NEEDLE DIRECTION

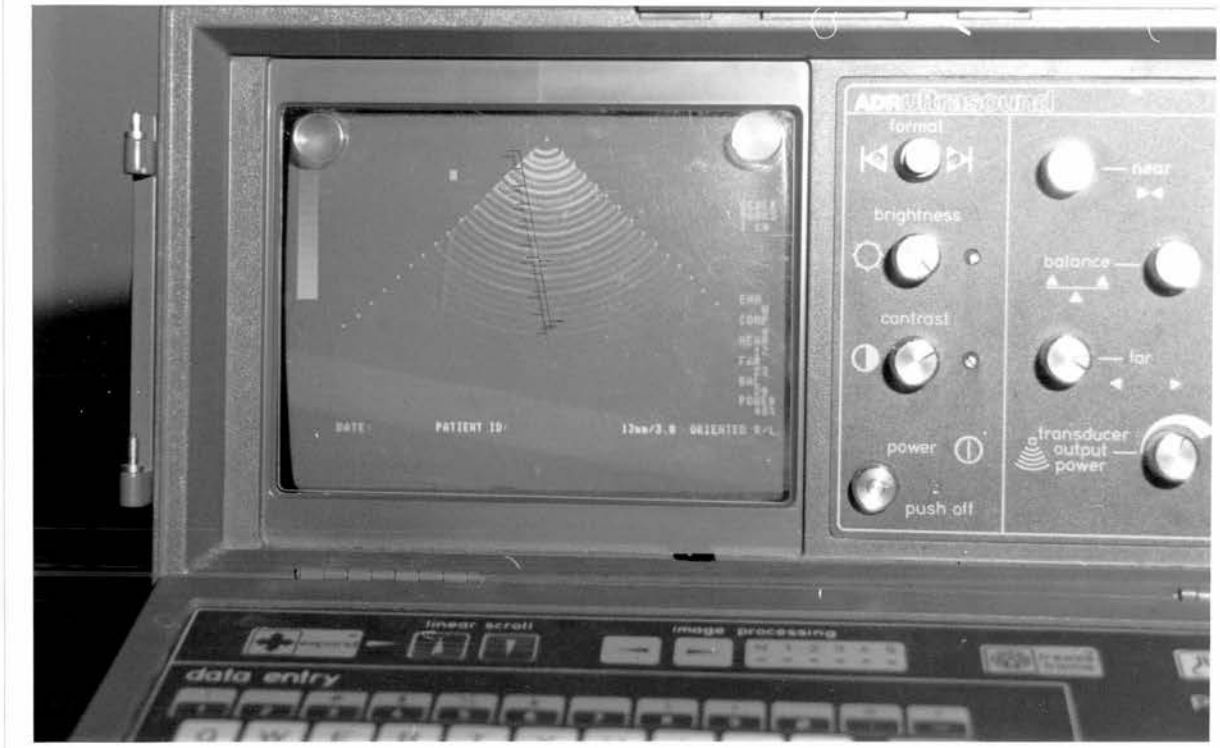


FIGURE 11: PERSPEX PLATE OVER ULTRASOUND MACHINE SCREEN

B) Data Recorded

The presence of a fetal heart, placental position and gestational age of the pregnancy were recorded prior to the chorionic villus sampling, and the fetal heart was also recorded after the chorionic villus sampling.

5.5 RESULTS

Villus was successfully recovered from 20 of 34 patients. The recovery rate appeared to improve with anterior placental positions compared with posterior placental positions, (Table 19).

5.6 DISCUSSION

The pilot study proved that the materials designed for transabdominal chorionic villus sampling using the ultrasound machine available allowed villus material to be obtained. The rate at which villi could be successfully aspirated appeared to be related to the placental position. For diagnostic chorionic villus sampling, aligning the uterus to the desired angle by regulating bladder filling might improve successful sampling from posterior placentae. In this pilot study altering uterine position by filling the bladder was not attempted in order to keep the time of the procedure (and general anaesthetic) to a minimum. One difficulty encountered was the method of aspiration. Holding the needle and transducer assembly with one hand, while attempting to pull out the plunger of the syringe attached to the needle with the other, proved clumsy. (In Denmark, Smidt-Jensen

TABLE 19

RESULTS OF THE PILOT STUDY

Placental position	Villi		Total
	Obtained	Not obtained	
Anterior	13	2	15
Posterior	7	12	19
Total	20	14	34

at that point usually sought help from a Radiographer who steadied the needle assembly while he used two hands for the aspiration).

An improvement in villus aspiration was designed which allowed the operator to have finger tip control of suction, while maintaining full control of the needle assembly.

A study comparing transabdominal chorionic villus sampling with transcervical chorionic villus sampling using the Aluminium cannula described in chapter 4 was performed.

A COMPARISON STUDY OF TRANSABDOMINAL AND TRANSCERVICAL CHORIONIC VILLUS SAMPLING

6.1 PATIENTS AND METHODS

Fifty five women, who gave informed consent, were entered into this study. Local ethical committee approval was obtained. Four of the 55 women were excluded because preliminary ultrasonography did not show the presence of a live fetus. One twin pregnancy was excluded.

The remaining 50 women had a single, live fetus with a gestational age as assessed by ultrasound measurements ranging from 7+ to 13 weeks (mean 9.5 ± 1.1 weeks).

6.2 EXPERIMENTAL DESIGN

I performed all the sampling and each sampling attempt was performed twice. Both the transabdominal and transcervical procedures were carried out under general anaesthesia immediately before therapeutic termination of pregnancy. In order to avoid moving the patient excessively while under general anaesthesia, the transabdominal chorionic villus sampling was always performed first. To alleviate any bias in villus recovery that might arise, the placental site was first localised by ultrasound prior to any sampling and both methods were used to sample only this allocation of placental site. Thus, in cases where placental localisation was uncertain, no bias was given to the transcervical procedure following the known outcome of the first (transabdominal) sampling.

6.3 ULTRASOUND EQUIPMENT

The ADR 4000 SL was used (section 5.3 A).

6.4 METHOD OF TRANSABDOMINAL CHORIONIC VILLUS SAMPLING

The method used for transabdominal chorionic villus sampling was identical to that which I had developed in the pilot study (section 5.4).

6.5 METHOD OF ASPIRATION FOR TRANSABDOMINAL CHORIONIC VILLUS SAMPLING

Suction was applied from wall suction connected by plastic tubing (Vygon, Lectro-Cath, 1.5 mm in diameter) to the 22 gauge needle hub via a paediatric mucous aspirator and suction was controlled by an R90 plastic connection in which a small hole had been drilled to allow finger-tip control of suction. The R90 connection was attached to the hub of the 22 gauge spinal needle (Figure 12).

6.6 METHOD OF TRANSCERVICAL CHORIONIC VILLUS SAMPLING

The method used was as described in section 4.5 B.

6.7 METHOD OF ASPIRATION FOR TRANSCERVICAL CHORIONIC VILLUS SAMPLING

Continuous suction, as in the transabdominal method, was applied (section 6.5).

6.8 CYTOGENETIC ANALYSIS

The villi were collected into sodium bicarbonate buffered RPMI (+5 I.U. Heparin per ml) and taken directly

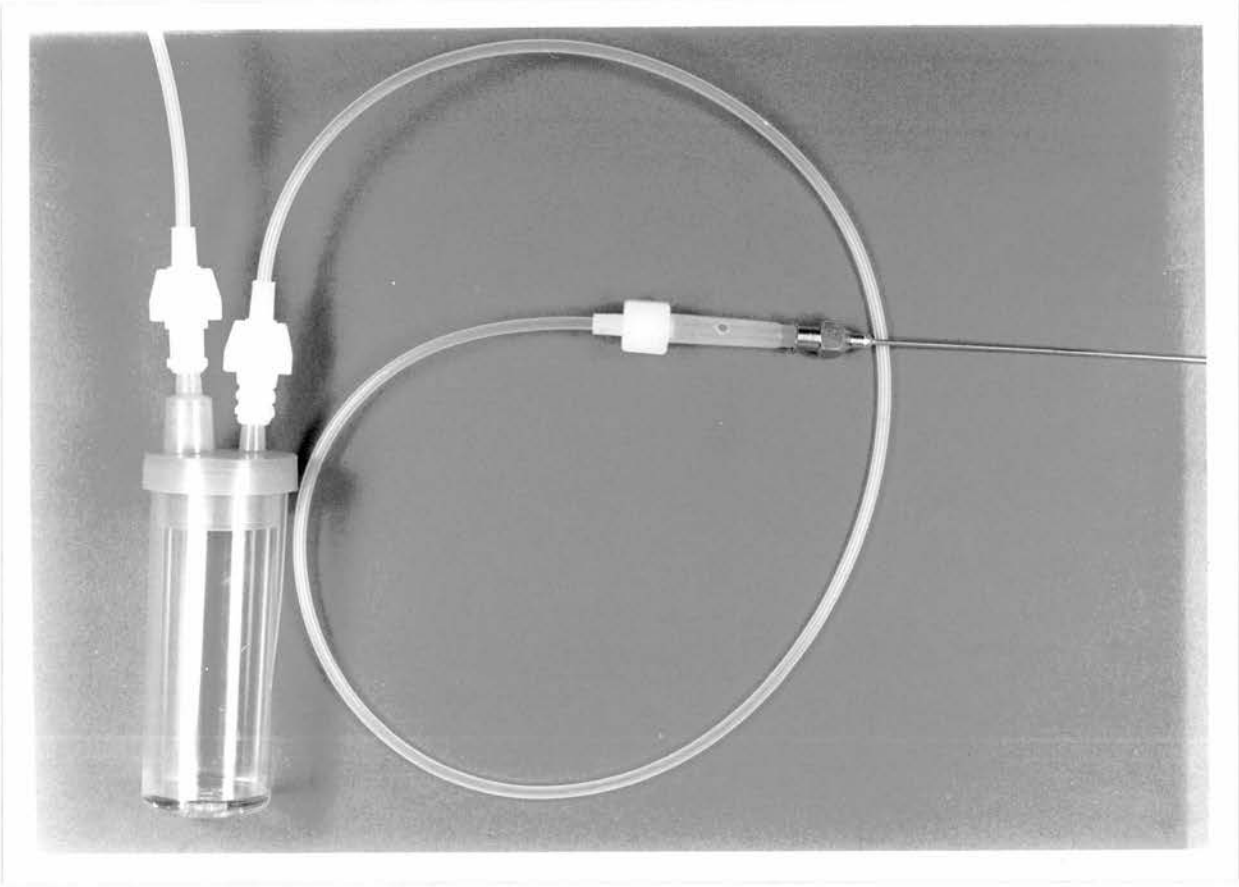


FIGURE 12: PAEDIATRIC MUCOUS ASPIRATOR CONNECTED VIA VYGON TUBING WITH R90 ADAPTOR (AND HOLE FOR FINGER CONTROL OF SUCTION) CONNECTED TO NEEDLE

to a laboratory where the villi were dissected out into fresh medium. The villus weights were estimated with reference to tubes containing 6 standard samples of villi of known weight (<5, 5, 5-10, 10, 10-20, >20 mgs). Ten milligrams of villi are the minimum required for reliable cytogenetic analysis, although 1 to 5 mgs of villi may also allow diagnosis, though unreliably, see Table 9, Chapter 4, Page 49.

6.9 RESULTS

The distribution of villus weights obtained by the transcervical and transabdominal methods are given in Table 20. When the two sampling methods were compared with respect to their ability to obtain at least 10 mgs of villi a highly significant difference was observed (X^2_M = 13.9, $p<0.001$)(McNemar test)(Figure 13). Adequate villus samples were obtained by transcervical chorionic villus sampling in 35 out of 50 (70%) of the patients, uner these study conditions, but in only 13 out of 50 (26%) of patients by the trans-abdominal approach. Gestational age was not found to be significantly different between successful and unsuccessful samples for both sampling methods. Figure 14 shows the distribution of villus weights obtained from 'anterior' and 'posterior' placentae where "success" was the proportion of patients in which an adequate sample was obtained. Four fundal placentae were recorded, and of these, three were assigned as 'anterior' as the bulk of the placenta was lying anteriorly. In this study no placentae were localised over the cervical os.

TABLE 20

VILLUS RECOVERY BY TRANSABDOMINAL AND TRANSCERVICAL CHORIONIC VILLUS SAMPLING (CVS)

Villus weights (mg)	Number of patients					
	Transabdominal CVS			Transcervical CVS		
	Posterior placenta	Anterior placenta	Total	Posterior placenta	Anterior placenta	Total
0	5	7	12	6	3	9
<5	5	5	10	1	0	1
5-<10	7	8	15	3	2	5
10-<20	7	4	11	5	3	8
20-<30	1	1	2	3	3	6
30-<40	0	0	0	5	8	13
40-<50	0	0	0	2	3	5
50-<60	0	0	0	0	2	2
60	0	0	0	0	1	1
Total no. patients	25	25	50	25	25	50
Approx. mean villus wt(mg)	6.6	5.4	6.0	15.7	26.5	21.1
S.D.	5.6	5.5	5.6	14.1	17.0	16.4
% adequate samples (>10 mg)	32.0	20.0	26.0	60.0	80.0	70.0

FIGURE 13

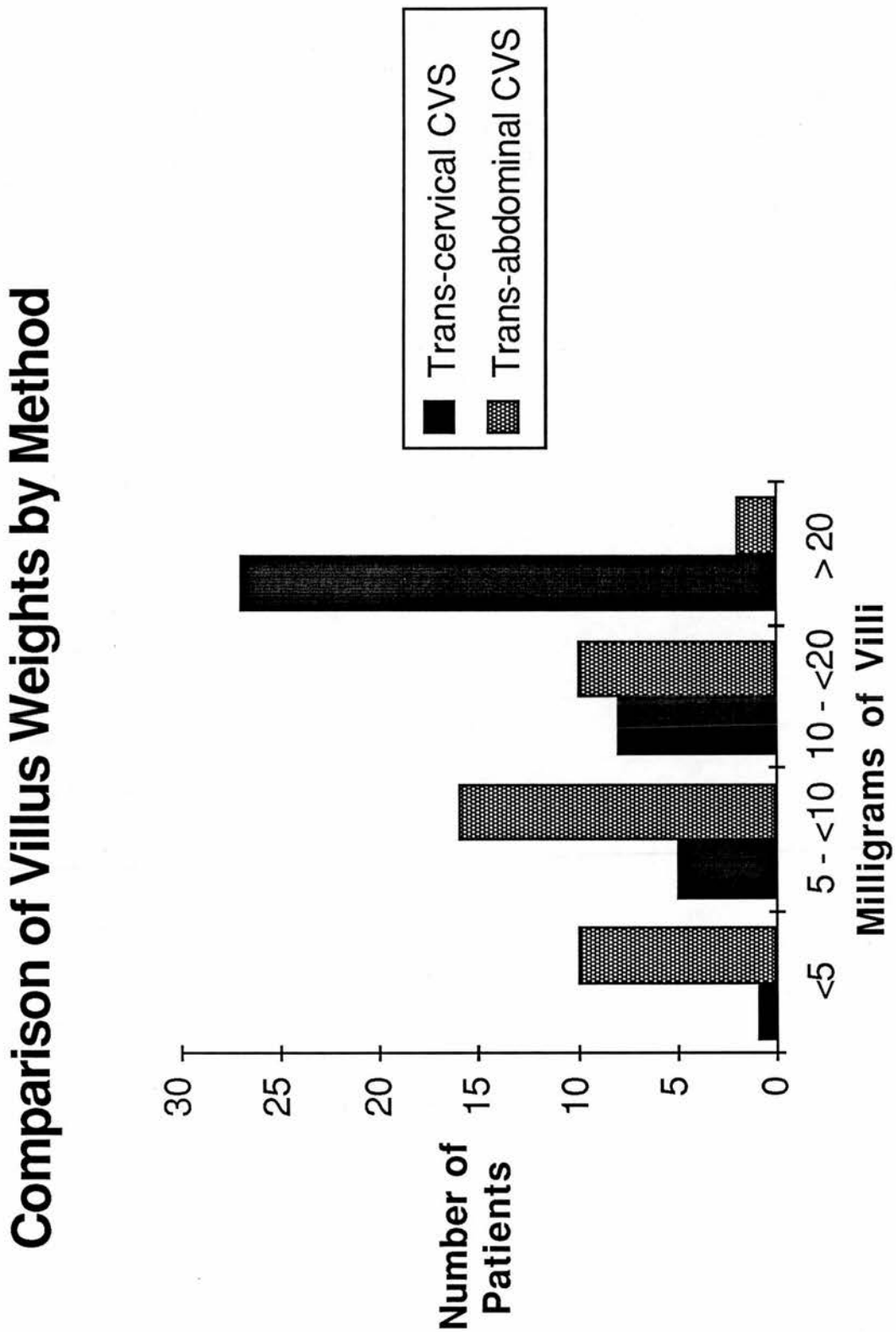
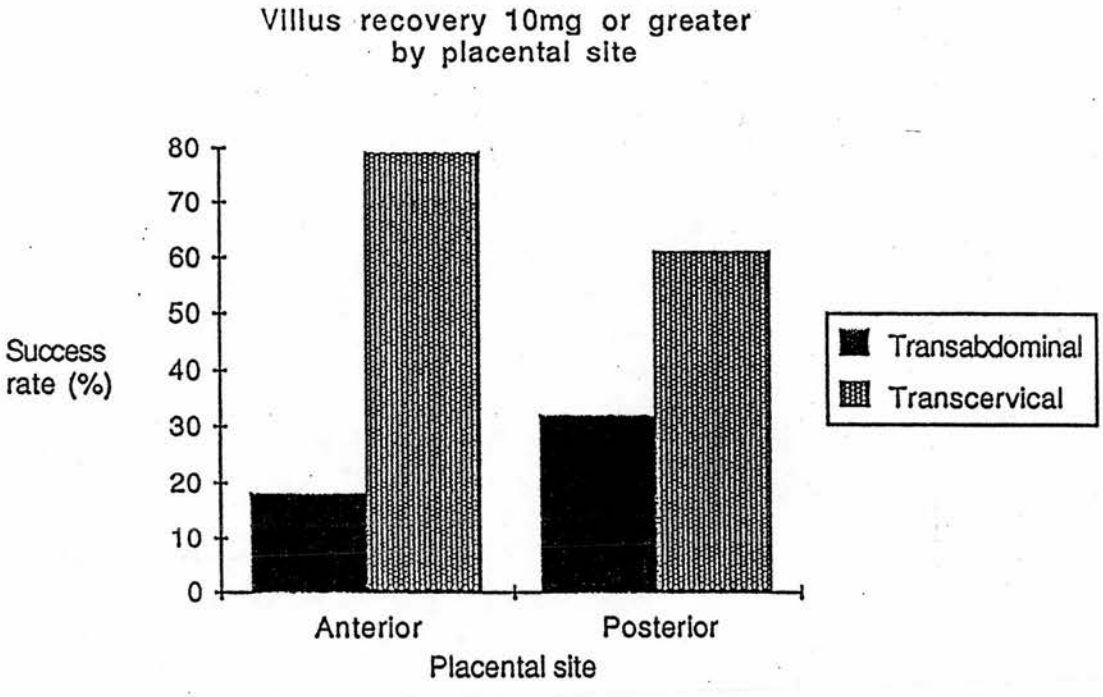

Comparison of Villus Weights by Method
Trans-cervical CVS
Trans-abdominal CVS
Number of Patients
0
5
10
15
20
25
30
<5
5 - <10
10 - <20
>20
Milligrams of Villi

FIGURE 14


Villus recovery 10mg or greater
by placental site
Success
rate (%)
80
70
60
50
40
30
20
10
0
Anterior
Posterior
Placental site
Transabdominal
Transcervical

The transabdominal technique obtained an adequate sample (10 mgs or greater) in 8 out of 25 posterior placentae and 5 out of 25 anterior placentae, compared with the transcervical method, where 20 out of 25 adequate samples were obtained when the placental site was anterior and 15 out of 25 when it was posterior (Table 20). However, the influence of the placental site on the success of these two methods is not significant ($X^2 = 1.3$, $p > 0.05$).

6.10 DISCUSSION

This study was designed to examine the efficacy of the transcervical method of chorionic villus sampling compared to a transabdominal method rather than an examination of the 'ideal' diagnostic situation, when there are no constraints on time and the optimal gestational age can be chosen prior to the chorionic villus sampling procedure. Transabdominal chorionic villus sampling using the technique described is characterised by lower weights of villi recovered, compared with the transcervical method. This is in agreement with previous findings (Bovicelli *et al* 1986; Brambati *et al* 1986). Brambati *et al* (1986) employed a larger bore needle, but the weight of villi obtained was still small, indicating that needle size does not seem to affect villus recovery. Prior to this study, I had gained experience with transabdominal chorionic villus sampling in the pilot study (section 5.2). My experience of transcervical chorionic villus sampling was greater, over 200. The numbers of patients in whom villi of any weight was obtained was not different

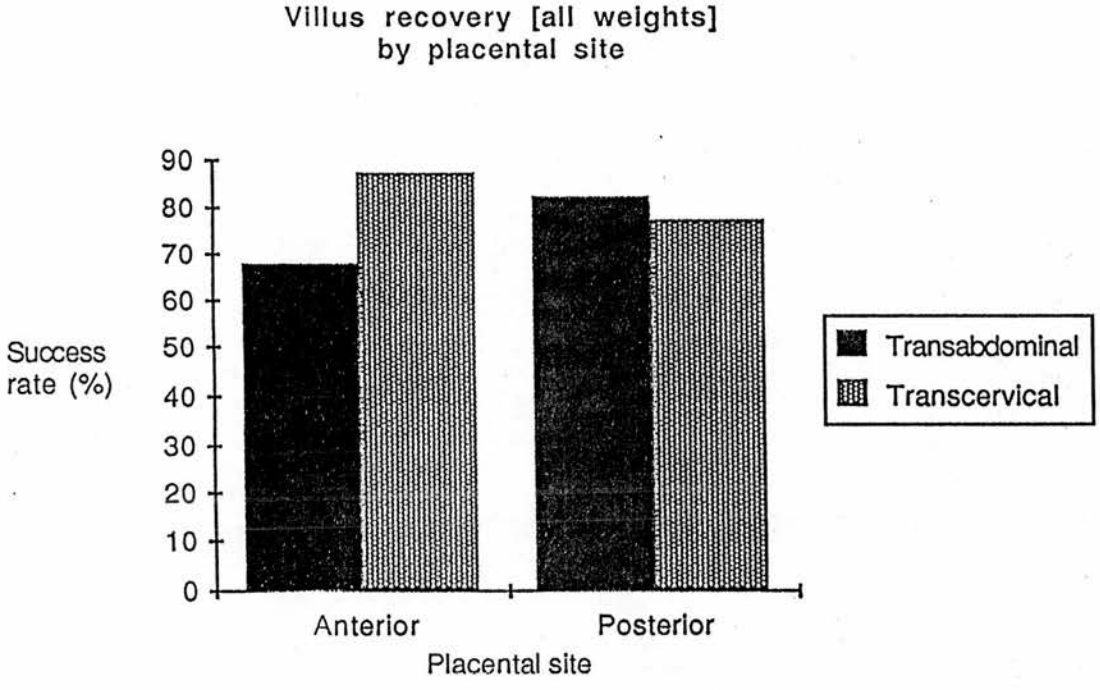
using either method of sampling in this study, thus the difference in experience of the operator using transcervical compared with transabdominal chorionic villus sampling does not appear to have affected overall villus recovery rates.

In this study, the time spent on both methods of sampling was the same, that is 30 to 60 seconds. However, in another report on transabdominal chorionic villus sampling, Smidt-Jensen et al (1986), the average time of suction was four minutes (1 min 20 sec to 15 min 40 sec). If a longer application of sampling time is a necessary requirement for satisfactory villus recovery by the transabdominal method then it might be expected to be more uncomfortable for the patient, and may, therefore, require some form of analgesia or local anaesthesia.

It was my expectation that a transabdominal approach would be more successful in obtaining chorionic villi from an 'anterior' placenta than sampling by a transcervical route, but there was no appreciable difference in obtaining access to either an 'anterior' or 'posterior' placenta by either method (Figure 15).

A summary of diagnostic series of chorionic villus sampling, as reported by Jackson (1986) shows a spontaneous miscarriage rate following transcervical chorionic villus sampling as being similar to that following transabdominal chorionic villus sampling. If this finding is confirmed by large randomised trials comparing the two techniques, then it is difficult to see any advantage for transabdominal chorionic villus sampling, especially for prenatal diagnosis by DNA or biochemical analysis, in which villus weights of

FIGURE 15


Villus recovery [all weights]
by placental site
Success
rate (%)
90
80
70
60
50
40
30
20
10
0
Anterior
Posterior
Placental site
Transabdominal
Transcervical

20 milligrams or more are necessary for reliable diagnoses (Upadhyaya *et al* 1984).

LABORATORY TECHNIQUES FOR CHROMOSOMAL ANALYSIS FROM CHORIONIC VILLI

7.1 INTRODUCTION

This chapter considers the value of chorionic villi for cytogenetic analysis and was derived from a collaboration between the author and the cytogenetic services.

Although published work existed on the use of chorionic villi for first trimester fetal diagnosis, for example, Hahnemann et al (1968), Kullander et al (1973) and Rhine et al (1979), it was not until the publication in 1981 by Niazi et al of a method of culturing chorionic villi and subsequent successful karyotype preparation that general interest in the use of chorionic villi for fetal karyotyping began. Prior to this, attempts at villus culture had failed because of the overgrowth in culture of maternal cells. Niazi et al (1981) overcame the problem of maternal cell contamination and the poor growth of villus cells in culture. Their method relied on a technique that removed the outer covering of the villus and exposed the mesenchymal core. The cells of this core were grown in culture. The cytotrophoblast and syncytiotrophoblast that covered the villus core were removed by trypsinisation followed by the passage of the trypsinised products through fine wire gauze which removed the maternal cell clusters normally adherent to the outer covering of the villi.

Simoni in 1983, when repeating this work still encountered maternal cell contamination (Simoni et al 1983).

In seeking an alternative to villus culture, they applied a direct karyotype preparation published in 1972 (Evans et al 1972). Using this direct method, trisomy 21 was successfully diagnosed in a fetus at eleven weeks gestation, five hours after chorionic villus sampling. It was this report that stimulated worldwide interest in the direct method of fetal karyotyping using chorionic villi obtained in the first trimester (Brambati et al 1983).

7.2 METHODS OF DIRECT CHROMOSOMAL ANALYSIS FROM CHORIONIC VILLI

The "direct" method first applied by Simoni et al (Table 21) relies on the presence within the cells of the cytotrophoblast (inner cell layer of the chorionic villus sheath) of spontaneous mitoses. Spontaneous mitoses constitute approximately one per cent of cytotrophoblastic tissue from first trimester chorionic villi (Watanabe et al 1978). Within chorionic villi there are many thousands of cytotrophoblastic cells and, therefore, several dozen mitoses would be present at any one time (Simoni et al 1986). These may be examined directly in metaphase without the need for tissue culture. Maternal cells which do not exhibit spontaneous mitoses, would then not interfere with the interpretation of metaphases obtained from villi and the problem of maternal cell contamination would be overcome.

Karyotype preparation using Simoni's technique was criticised by Burgoyne (Burgoyne 1983) because there was a low mitotic index and poor chromosome morphology as well as

TABLE 21

DIRECT METHOD OF KARYOTYPE PREPARATION FROM CHORIONIC VILLI

Simoni et al (1983)

1. Villi are selected and put into a 60 mm Petri dish containing 3 mls of RPMI medium without serum.
2. Colcemid is added to the medium to reach a 0.04 µg/ml final concentration. The villi are left for one hour at room temperature.
3. The medium is removed and replaced with 5 mls of 1% sodium citrate solution for hypotonic treatment for 10 minutes.
4. The hypotonic solution is removed and 5 mls of methanol-acetic acid 3:1 fixative is added for 10 minutes.
5. The fixative is aspirated and replaced with 3 mls of aqueous 60% acetic acid.
6. After 3-5 minutes, 3 mls of methanol is added.
7. The cell suspension is transferred into a tube and centrifuged in 0.3 ml of methanol-acetic acid 1:1 fixative.
8. Using warmed slides (40° - 50°C), the cell suspension is dropped on to each slide and distributed on the surface of the slide by means of a bent Pasteur pipette. The slides are air dried for 30 minutes at (40° - 50°C) before staining. Q.F.Q. banding is used.

many broken mitoses. The implications of this for a diagnostic cytogenetic service would be that only gross numerical abnormalities could be detected using Simoni's method.

The method of Burgoyne proved to be easier and the metaphases so obtained were amenable to G-banding (Appendix 3). Simoni's technique had always used Q.F.Q. banding which is a technique not widely used among Cytogenticists in the United Kingdom. Burgoyne's technique has been adapted by Dr T Webb of the Clinical Genetics Department, Birmingham Maternity Hospital (Table 22) and was the method used for karyotype preparation from villus samples obtained in Chapter 4.

The essential steps of these methods are contrasted in Table 23.

A) Application of Webb's Method of Direct Karyotype Preparation

(i) Patients

Fifteen patients who had transcervical chorionic villus sampling performed as outlined in section 4.5 B were used. A minimum of 10 mgs of villi was obtained from each.

(ii) Method

The method of karyotype preparation is described in Table 22. One slide per patient was selected and examined, and the metaphases obtained were categorised as follows:-

TABLE 22

DIRECT METHOD OF KARYOTYPE PREPARATION FROM CHORIONIC VILLI

Webb after Burgoyne (1983)

1. The villus sample is placed in a tube containing 5 mls of medium (Hams F10 with 20% fetal calf serum, glutamine, penicillin and streptomycin), at approximately 37°C and transported to the laboratory.
2. The villi are transferred into a dish and examined under an inverted light microscope when the quantity and quality of the villi are recorded with reference to villus of standard weights. Maternal debris, blood clot and decidua are dissected free with fine forceps from the villi. The sample is transferred back into a tube.
3. Two drops of colcemid (approximately 0.1 ml (1 µg/ml)) are added to each sample which is then incubated at 37°C in a water bath for one hour.
4. The villi are then spun down (5 minutes at 1000 r.p.m.) and the medium (and colcemid) removed.
5. Approximately 5 mls of 1% sodium citrate solution is added to the villi and whirlimixed. The samples are incubated for a further 25 minutes (at 37°C).
6. The villi are spun down (5 minutes at 1000 r.p.m.) and the supernatant removed.
7. Approximately 5 mls of fixative (3 methanol:1 glacial acetic acid) are added slowly whilst the samples are agitated for 30 seconds in a whirlimix.

8. The villi are spun down (5 minutes at 1000 r.p.m.) and the fixative replaced.
9. The samples are stored in a freezer (at -20°C) overnight to ensure complete fixation.
10. The villi are spun down (5 minutes at 1000 r.p.m.) and the fixative removed.
11. 5-10 drops of 60% acetic acid are added and the tube agitated for 30 seconds.
12. 5 mls of fresh fixative are added to stop the action of the acetic acid.
13. The cells are spun down and the fixative replaced twice.
14. The cells are resuspended in a few drops of the fixative and dropped on to cold clean slides and left to air dry (about 20 minutes).
15. G-banding is then performed using the method outlined in Appendix 3.
16. When dry, the slides are made permanent and can then be examined under the light microscope.

TABLE 23

THE THREE METHODS OF DIRECT KARYOTYPE PREPARATION COMPARED

Steps	Simoni et al	Webb (after Burgoyne)	Flori et al	Comment
1	Clean villi	Clean villi	Clean villi	Removes maternal contaminations
2	Colchecine	Colcemid	Colchecine	Inhibits cell division at metaphase
3	Sodium citrate	Sodium citrate	Sodium citrate	Hypotonic cells swell and chromosomes separate
4	10 minutes in fixative	24 hours in fixative	24 hours in fixative	Preservation of tissue and inhibition of bacterial and fungal contamination
5	-	-	Resuspension in increasing concentrations of alcohol	Slow rehydration of cells and fixative removal
6	60% acetic acid	60% acetic acid	60% acetic acid	Cell dissociation and release of cellular contents into solution
7	Fixative added	Fixative added	-	-
8	Cell spreading with bent Pasteur pipette on warmed slides	Cell spreading by dropping solution from a distance onto cold slides	Evans et al drop/re-drop method Hot slides	Chromosome spreading onto slides
9	Q.F.Q. banding	G-banding	G-banding	Identification of individual chromosomes

ANALYSABLE	suitable for numerical and/or banding analysis (Figure 16)
POOR	not suitable for analysis due to poor chromosome morphology and/or overlapping chromosomes
BROKEN	obviously broken metaphases

(iii) Results

Table 24 gives the results for the 15 patients.

(iv) Discussion

Less then 10 per cent of the total number of cells in metaphase were analysable and the remainder were poor or "broken".

The quality of chromosomes obtained using this method was inferior to those karyotypes obtained from amniotic fluid cultures (Figures 17 and 18). It was felt that particular stages in the technique could have resulted in the high number of broken mitoses and poor metaphase spreads. Flori in 1985, described a modification of the direct chromosome technique in which the villi were rehydrated in stages using first 100% alcohol, then 70% alcohol, then 50% alcohol and finally water and using this technique, it was found that the number of interpretable metaphases obtained increased by a factor of five compared with Simoni's technique (Flori et al 1985). This rehydration step was introduced to the direct method in use and the method of making slides revised (Evans 1972). The final method, known as the "Flori" technique is given in Table 25.

FIGURE 16: ANALYSABLE METAPHASES USING A DIRECT PREPARATION FROM A CHORIONIC VILLUS SAMPLE (KARYOTYPE 46,XX)

TABLE 24

RESULTS OF DIRECT KARYOTYPE PREPARATION USING WEBB'S MODIFICATION OF BURGOYNE

Patient	Quality of Metaphases			
	Analysable	Poor	Broken	Total
1	0	1	0	1
2	3	24	13	40
3	0	0	0	0
4	7	8	20	35
5	5	44	7	56
6	0	16	5	21
7	0	0	0	0
8	0	5	4	9
9	0	1	3	4
10	0	0	0	0
11	0	0	0	0
12	0	0	1	1
13	1	1	3	5
14	3	19	4	26
15	1	2	4	7
Total	20	121	64	205
Mean	1.3	8.1	4.3	13.7
Standard deviation	2.2	12.7	5.6	17.8
Total of each metaphase category as a % of total	9.8	59.0	31.2	

COMPARISON OF CHROMOSOME PREPARATIONS

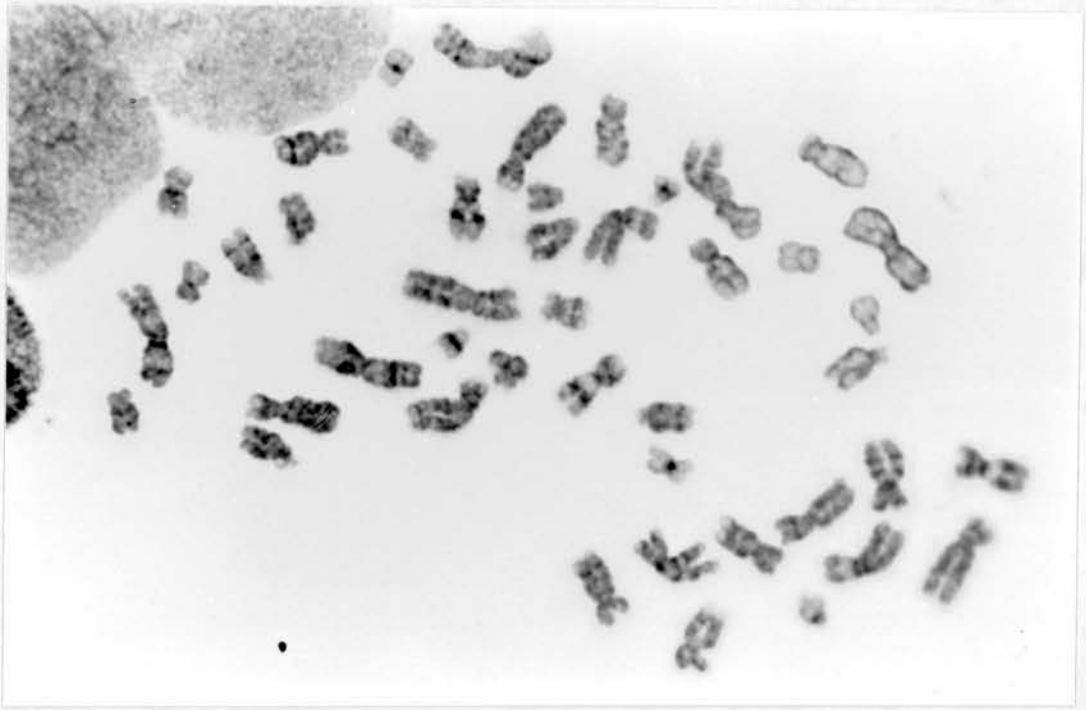


FIGURE 17: CHORIONIC VILLUS SAMPLE DIRECT PREPARATION

FIGURE 18: AMNIOTIC FLUID CULTURE

TABLE 25

MODIFIED TECHNIQUE FOR DIRECT KARYOTYPE PREPARATION

After Flori et al (1985)

1. Chorionic villus samples are collected in RPMI 1640 medium with 5 IU/ml heparin 0.1 mM/ml glutamine, 100 IU/ml penicillin and 100 µg/ml streptomycin and buffered with sodium bicarbonate.
2. The sample is examined using a dissecting microscope and the villi dissected out and washed in RPMI 1640 medium without heparin and 10% fetal calf serum (FCS).
3. 0.1 ml colcemid (10 µg/ml) was added and the villi incubated at 37°C for 1 hour.
4. The villi are centrifuged at 1000 r.p.m. for 5 minutes and the supernatant discarded and replaced with 5 mls prewarmed 1% sodium citrate solution for 20 minutes at 37°C.
5. This is removed after centrifugation and the villi are fixed in 10 mls 3:1, absolute methanol: glacial acetic acid. The fixative is changed and the tubes placed in a deep freeze (-20°C) for at least 45 minutes before slide preparation.
6. Before dissociation the villi were rehydrated in stages. The fixative was removed after centrifugation and approximately 3 mls methanol added, then deionised water is added 1 ml at a time to a concentration of 50%. A further 10 mls of water was added after the removal of

the methanol/water after centrifugation at 2000 r.p.m. for 5 minutes. The villi were centrifuged once more and the supernatant poured off and 10 drops of 70% acetic acid added to the tube.

7. After 4 minutes slides are prepared by placing one drop of cell suspension at one end of each slide and immediately sucking it up and placing it adjacent to the first drop and again sucking it up immediately, this is repeated three more times (Evans et al 1972). Slides are warmed on a hot plate at approximately 45°C.
8. The slides are G-banded after 'ageing' overnight in an oven at 50°C, as outlined in Appendix 3.

B) Application of "Flori's" Method of Direct Karyotype Preparation

(i) Patients

Eighteen patients, who formed part of the study reported in section 7.5, were used. The method of chorionic villus sampling was that described in sections 4.5 B and 6.5.

(ii) Method

One slide per patient was examined in order to be consistent with previous data using Webb's technique. The metaphase spreads were categorised as previously outlined.

(iii) Results

Table 26 shows the results from the 18 patients. Table 27 compares Webb's with "Flori's" technique.

(iv) Discussion

Using the method of "Flori", the number of analysable divisions has substantially increased as have the total number of divisions (from 10 to 23%). The "Flori" technique also results in relatively less "poor" divisions (from 59 to 37%) but more broken divisions (from 31 to 40%), so that if the technique is to be improved, ways must be found of reducing the breakage of mitotic spreads.

TABLE 26

RESULTS OF DIRECT KARYOTYPE PREPARATION ("FLORI" METHOD)

Patient	Quality of Metaphases			
	Analysable	Poor	Broken	Total
1	2	1	0	3
2	0	0	1	1
3	10	13	22	45
4	8	15	39	62
5	11	19	17	47
6	8	16	11	35
7	10	9	4	23
8	13	18	20	51
9	6	24	13	43
10	6	12	20	38
11	7	7	3	17
12	18	25	20	63
13	3	9	2	14
14	4	6	6	16
15	8	9	18	35
16	2	5	2	9
17	8	19	25	52
18	12	15	15	42
Total	136	222	239	596
Mean	7.6	12.3	13.3	33.1
Standard deviation	4.5	7.2	10.5	19.5
Total of each metaphase category as a % of total	22.8	37.3	40.1	

TABLE 27

MEAN NUMBER OF DIVISIONS PER SLIDE

Method	Analysable	Poor	Broken	Total
Webb	1.3	8.1	4.3	13.7
"Flori"	7.6	12.3	13.3	33.1

PROPORTIONS OF DIVISIONS PER SLIDE (AS % OF TOTAL)

Method	Analysable	Poor	Broken
Webb	9.8	59.0	31.2
"Flori"	22.8	37.3	40.1

7.3 SHORT TERM CULTURE OF CHORIONIC VILLI

It was purported that whole villi incubated in medium at 37°C for 1-2 days and then prepared by a direct method had improved chromosome morphology, thus reducing the time spent by the laboratory in examining the material.

There have been few reports where a direct preparation and a short term culture method for processing chorionic villi have been compared. Figures are presented in Terzoli et al 1985, that indicate a peak in the number of mitoses between 49 and 52 hours incubation. Stewart et al 1986, have reported that although there is a slight decrease in the average number of metaphases after short term incubation compared to direct preparation the chromosomal morphology was improved after short term incubation. However, in Stewart et al 1986, no figures were given and in Terzoli et al 1985, the quality of the metaphases examined was not reported.

7.4 A COMPARISON STUDY BETWEEN "DIRECT" AND SHORT TERM CULTURE

A) Introduction

A study was conducted comparing the direct method of Flori, Table 25, with a 24 hour and 48 hour short term culture for chorionic villi, to assess which method was best in terms of the quality of metaphase spreads obtained that would be useful for fetal karyotyping.

B) Patients

Twenty patients undergoing transcervical chorionic villus sampling for diagnostic purposes were used. Their gestational ages ranged from 9 to 12 weeks.

Chorionic villi were collected via a transcervical technique using the Aluminium cannula by the method described in section 4.5 B.

C) Methods

The method of karyotype preparation was that outlined in Table 25.

Ten to fifteen milligrams of villi were placed in 5 mls of RPMI with 10% Fetal Calf Serum (F.C.S.) in each of 3 tubes.

The villi for the 24 hour and 48 hour short term cultures were incubated at 37°C before harvesting and those for direct preparations were processed immediately.

0.1 ml colcemid (1µg/ml) was added for 1 hour (direct preparations) or 2 hour (short term cultures).

Slides were G-banded (Appendix 3) after "ageing" overnight at 60°C. For the purposes of this study, two slides were scanned from each method per patient and all metaphases were categorised as outlined in section 7.2 A (ii).

D) Analysis of Data

The data were analysed following $\sqrt{Y}$ transformation by a two-dimensional, mixed model analysis of variance. This test allows greater discrimination between variables than alternative tests, for example, a paired t test (Sokal *et al* 1981). The

"methods" were considered to be a fixed effect and the "patients" and replicates as random effects. The F values (Tables 28 and 29) are variance ratios obtained by dividing the mean squares of the "methods" or "patients" terms by the interaction. The interaction term (Tables 28, 29 and 30) arises due to the effect of the "patients" on the outcome of the "methods". The variances (Table 30) are calculated from the mixed model ANOVAR and the proportions of each in relation to the total variation are given as a percentage.

E) Results

The results show that the mean number of analysable divisions per slide ranges from 6.05 in samples prepared by the direct method to 3.30 following a "48 hour" incubation (Table 31). The variation about these means, however, is substantial and the differences between the methods are found to be not significant. There is also no significant difference between "patients" (Table 28 (i)). The third component of variation, the interaction term, is highly significant (Table 28 (i)) and accounts for 64.8% of all the observed variation (Table 30).

The interpretation of this, is that either

A. different patients respond more favourably to different methods

OR

B. that it is the random distribution of 'good quality' villi, destined to produce good quality metaphases, within the three tubes set up for the three methods, that

TABLE 28

AN ANALYSIS OF THE VARIATION FROM DIRECT AND SHORT TERM CULTURE METHODS

SOURCE OF VARIATION	Degrees of freedom	(i) ANALYSABLE		(ii) POOR		(iii) BROKEN		(iv) TOTAL NUMBER OF DIVISIONS	
		F	p	F	p	F	p	F	p
methods	2	2.10	n.s.	7.14	**	11.68	**	7.25	**
patient	19	0.88	n.s.	1.77	n.s.	1.89	n.s.	1.19	n.s.
interaction	38	5.20	**	4.95	**	4.01	**	7.50	**
residual	60								
total	119								

n.s. $p>0.05$, not significant

** $p<0.01$

Data transformed by $\sqrt{Y}$.

TABLE 29

AN ANALYSIS OF THE VARIATION WITHIN SAMPLES PREPARED AFTER A "24 HOUR" INCUBATION

		PREDICTED RESULTS		OBSERVED RESULTS							
		(i)	(ii)	(iii) ANALYSABLE		(iv) POOR		(v) BROKEN		(vi) TOTAL NUMBER OF DIVISIONS	
SOURCE OF VARIATION	Degrees of freedom	A	B	F	p	F	p	F	p	F	p
methods	2	n.s.	n.s.	2.69	n.s.	0.05	n.s.	3.03	n.s.	1.91	n.s.
patient	19	**	n.s.	2.57	**	3.63	**	4.97	**	4.54	**
interaction	38	n.s.	**	3.91	**	3.65	**	2.77	**	4.62	**
residual	60										
total	119										

n.s. $p>0.05$, not significant

** $p<0.01$

Data transformed by $\sqrt{Y}$.

TABLE 30

THE VARIATION DUE TO THE "METHODS", "PATIENTS", AND INTERACTION TERMS

SOURCE OF VARIATION	STUDY 7.4 VARIATION	STUDY 7.5 VARIATION
methods	0.07 (4.6)	0.05 (4.3)
patients	0.00 (0.0)	0.33 (28.5)
interaction	0.98 (64.5)	0.46 (39.7)
residual	0.47 (30.9)	0.32 (27.6)
total	1.52	1.16

Variation as a % of the total is given in brackets

TABLE 31

THE NUMBER OF METAPHASE CELLS IN CHORIONIC VILLI PREPARED BY DIRECT AND SHORT TERM CULTURE METHOD

	DIRECT	"24 hour"	"48 hour"
ANALYSABLE			
Mean number*	6.05	4.78	3.33
Total number	242	191	133
Proportion (%)	23.11	30.41	36.44
POOR			
Mean number*	11.10	6.38	3.95
Total number	443	255	158
Proportion (%)	42.31	40.61	43.29
BROKEN			
Mean number*	9.05	4.55	1.85
Total number	362	182	74
Proportion (%)	34.57	28.98	20.27
TOTAL NUMBER OF DIVISIONS			
Mean number*	26.17	15.70	9.13
Total number	1047	628	365

* per slide, 20 patients, 2 slides per sample

leads to some patients apparently responding more favourably to one method compared to another.

If the sample from any one patient responds differently with respect to the three cytogenetic methods, then for any diagnostic sample for fetal karyotype analysis, a direct and short term culture should be set up. If, however, it is the random distribution of 'good quality' villi between the methods that leads to the highly significant interaction term, then for any diagnostic case, whichever method is convenient for the laboratory can be used.

In order to test which of these hypotheses, A or B, was applicable, a further study applying one method to each of 3 tubes containing villi from a single patient was performed.

7.5 APPLICATION OF A SINGLE METHOD OF KARYOTYPE PREPARATION TO A SPLIT SAMPLE OF CHORIONIC VILLI

A) Patients

Twenty pre-termination patients (8-12 weeks gestation) had transcervical chorionic villus sampling performed as outlined in section 4.5 B. In all cases formal consent was given.

B) Method

The method of collection and karyotype preparation was that given in Table 25. The sample of villi from each patient was divided into 3 tubes with 10 - 15 mgs of villi in each tube.

C) Results

Table 32 shows the number of metaphases obtained in each of the 3 tubes prepared by a single method. If hypothesis A was correct then one would expect the "methods" and interaction terms to be not significant and the "patient" variation to be significant. If hypothesis B was correct, one would predict both the "method" and "patient" effects to be not significant but the interaction term would remain significant (Table 29 (i) and (ii)). An analysis of the number of analysable divisions is given in Table 29 (iii) and shows significant "patient" ($p<0.01$) and interaction terms ($p<0.01$), which fits with neither model precisely. These two components account for 28% and 40% of the total experimental variation, respectively (Table 30).

7.6 DISCUSSION OF STUDIES 7.4 AND 7.5

When making a cytogenetic assessment of a laboratory technique, it is the number of analysable cell divisions obtained rather than the total number of divisions that are important. In the study 7.4, more analysable divisions were present per slide than in the results from study 7.5 (Tables 31 and 32). This may be due to the less than ideal circumstances under which the pre-termination specimens were obtained and to the disparity between the average gestational age of the samples (10 - 11 weeks in the first study 7.4 and 8 - 9 weeks in the second study 7.5).

The variation observed between the samples in 7.4 may be due to three factors: an effect of the methods, variation arising from the random allocation of different quality villi

TABLE 32

THE NUMBER OF METAPHASE CELLS IN CHORIONIC VILLI PREPARED BY DIRECT CULTURE METHOD

	TUBES		
	1	2	3
ANALYSABLE			
Mean number*	3.60	3.05	2.05
Total number	144	122	82
Proportion (%)	25.35	22.30	20.71
POOR			
Mean number*	6.13	6.48	5.48
Total number	245	259	219
Proportion (%)	43.13	47.35	55.30
BROKEN			
Mean number*	4.48	4.15	2.38
Total number	179	166	95
Proportion (%)	31.51	30.35	23.99
TOTAL NUMBER OF DIVISIONS			
Mean number*	14.20	13.68	9.90
Total number	568	547	396

* per slide, 20 patients, 2 slides per sample

between tubes (hypothesis B) and a difference between the "patients" in their response to a method (hypothesis A).

The analysis of the data from 7.4 shows no significant difference between the three methods. An apparent trend is observed in the summed data in Table 31 with higher numbers of analysable, poor and broken metaphase cells in villi prepared by the direct method compared to the short term culture methods. This is similarly observed between the three tubes in 7.5 (Table 32), even though they were all processed by the same method. Thus, if there is any advantage in using one of these methods, it is small. Terzoli et al (1985) examined 8 villus specimens over a range of incubation times (2 hours to 52 hours) and observed a relatively synchronised burst of mitoses occuring after 49 hours incubation. In this study, the incubation times did not exceed 45 hours which may account for there being no corresponding increase in mitotic index in the samples prepared by the "48 hour" culture method in 7.4.

Following 7.4, two models were proposed as possible explanations of the interaction between the "patients" and the "methods". The results from 7.5 do not confirm either hypothesis A or hypothesis B, and the data (Table 29) indicates that the observed experimental variation may therefore be composed of both random "within a patient" variation (hypothesis B) and differences between "patients" in their response to a technique (hypothesis A)(Tables 29 (i), (ii) and (iii)).

The largest contributing factor to the observed variation, when comparing these methods, appears to be a random within

"patient" variation (hypothesis B), which accounted for approximately 40% of the total variation 7.5 (Table 30). This variation may be due to the physiological differences known to occur between the villi within a placenta (Kaufmann 1982). Upadhyaya et al (1987) recently examined the gross morphology of villi within placentae in relation to the sampling site and the diagnostic result. Within a placenta they recognised two villus types; a stringy form and a budding form. Their data shows that in direct preparations the presence of budding villi was associated with adequate to very good preparations and the stringy villi tended to produce poor preparations.

The variation that may be attributed to the difference between the "patients" in their response to the methods (hypothesis A) and which generated 28% of the total variation recorded in 7.5, may also be due to gross physiological differences. The villi at the periphery of the placenta are known to degenerate and so it is not unreasonable to expect the sampling site to affect the quality of a whole sample. The stage of development of the placenta may also affect the type of villi obtained, for example, the gestational ages of the samples in these studies range from 8 to 12 weeks. The data presented from the study by Terzoli et al (1985) illustrates similar variation between the "patients" in their response to a treatment. Following incubation, a doubling of the mitotic index was found to occur between 49 and 52 hours incubation, but only 4 of the 8 samples peaked within one time period (50 hours). Care must be shown when

interpreting the findings of studies comparing cytogenetic methods where this "patient"/"method" interaction has not been taken into account.

Cytogenetic laboratories should accept that differences in the quality of the villi in any one placenta will result in unpredictability in processing this material and it may be that all direct and short term culture methods, which rely on the naturally occuring mitotic index, will be inconsistent. Given the occurence of a difference between the patients in their response to a method, it appears that where sufficient villi and staffing resources are available, it would be advisable to prepare the villus samples by more than one direct or short term culture method.

Using the methods outlined in these studies, there has been 100% successful karyotyping from chorionic villus samples, outlined in Chapter 8.

A DIAGNOSTIC SERIES OF FIRST TRIMESTER CHORIONIC VILLUS SAMPLING PROCEDURES

8.1 A) INTRODUCTION

This chapter describes one hundred consecutive patients who had diagnostic chorionic villus sampling performed between 1985 and 1987, using the techniques given in detail in chapters 4, 5 and 6. Where karyotype analysis was performed, the laboratory techniques described in chapter 7 were used. All chorionic villus sampling procedures were carried out by me at the Birmingham Maternity Hospital. This hospital is situated in Central Birmingham Health District, one of twenty two districts, comprising the West Midlands Regional Health Authority. This, the largest Health Authority in the United Kingdom, serves a population of nearly six million people. The patients described in this chapter were referred from throughout the West Midlands Regional Health Authority and in some cases from beyond its boundaries.

Although there has been a screening programme for certain fetal abnormalities using amniocentesis (since 1976) and serum alpha-fetoprotein blood testing (since 1981), the West Midlands Regional Health Authority has one of the lowest rates of prenatal screening among Health Authorities in England and Wales. In 1986, 2% of pregnancies had prenatal screening in the West Midlands compared with 5% of pregnancies in the Trent, Wessex or Mersey Regional Health Authority areas (ACC 1986).

A low uptake of prenatal screening services may be due to lack of public knowledge of what is available and late presentation for such tests, but may also be due to

lack of awareness by medical personnel of which screening tests are available and which patients are eligible.

The aim in setting up diagnostic chorionic villus sampling was to successfully diagnose fetal abnormality before twelve weeks gestation, such that where termination of pregnancy was found to be indicated it could be performed before thirteen weeks gestation. The benefits of such an approach have already been described in section 3.1.

Faced with a background of low uptake of existing prenatal diagnostic tests where the timespan for testing was relatively long (amniocentesis and ultrasound), I realised that the take up of chorionic villus sampling, in which the timespan for testing was short (9 - 11+ weeks gestation), might be even less because of the late presentation of many pregnant patients to their doctor. An important part of setting up diagnostic chorionic villus sampling as a prenatal test would be increasing awareness of the test by the public and also those professionals involved in the health care of pregnant women.

8.1 B) INCREASING PUBLIC AWARENESS OF CHORIONIC VILLUS SAMPLING

By contacting journalists, several articles on prenatal diagnosis and chorionic villus sampling appeared in local Birmingham newspapers in 1985 and 1986. This was followed by two news items on chorionic villus sampling put out on local television throughout the Region.

Those families known to the Regional Clinical Genetics Services to be at risk of congenital diseases for which chorionic villus sampling could be a useful prenatal screening test, were made aware that a new test was available but early attendance in pregnancy was important. Efforts were made to obtain blood for DNA analysis from children with Cystic Fibrosis and their families such that, when pregnancies occurred in such families, DNA analysis for prenatal diagnosis would be possible.

Using the Muscular Dystrophy Register (held in the Department of Clinical Genetics), families who had expressed an interest in fetal sexing and chorionic villus sampling in any future pregnancy were also contacted about early attendance in pregnancy.

8.1 C) INCREASING MEDICAL PERSONNEL'S AWARENESS OF CHORIONIC VILLUS SAMPLING

In 1985, a Chorionic Villus Sampling Study Group which met monthly in 1985 and quarterly from 1986, was set up. Its function was to bring together the medical and laboratory workers involved in chorionic villus sampling research in Birmingham. It consisted of four Obstetricians, the two Regional Clinical Genetics Consultants, the two Consultant Cytogeneticists from the two Regional laboratories in Birmingham, as well as those laboratory workers and field workers directly involved in prenatal screening.

The group fostered research into chorionic villus sampling as well as critically evaluating the results of such work. It appraised articles on chorionic villus

sampling in the medical literature. It produced a document explaining the techniques of chorionic villus sampling and outlining the work on this subject being carried out in Birmingham, as well as indicating the method of referral of eligible patients for this test.

This document was circulated to all Consultant Obstetricians in the West Midlands Regional Health Authority area and later to all General Practitioners in the area. Throughout the years 1985 to 1987, lectures on chorionic villus sampling were given to interested health personnel throughout the West Midlands and in December 1985, the Second International Conference on Chorionic Villus Sampling and Early Prenatal Diagnosis was hosted in Birmingham.

8.2 ORGANISATION AND PROCEDURES OF THE CHORIONIC VILLUS SAMPLING CLINIC

A) Patient Referral

One hundred patients are described, who were referred from throughout the West Midlands Regional Health Authority area.

Forty-two patients had chorionic villus sampling performed where the sole indication was maternal age. By 1986, Regional policy was to offer prenatal testing for chromosomal abnormalities to women of 37 years or over but by August 1987, this had dropped to 35 years. Thirty-seven patients had been randomised to receive chorionic villus sampling as part of the Medical Research Council (MRC) Trial comparing amniocentesis with chorionic villus sampling. All these women were referred via their General Practitioners and in some cases, their Obstetricians.

The Regional Clinical Genetics Service referred three women with familial translocations, fourteen women for fetal sexing (one patient had chorionic villus sampling performed in successive pregnancies) and twelve women for fetal DNA analysis.

Twenty-six women, who had had a fetus or child with a chromosomal abnormality, were referred from Obstetricians and the Regional Clinical Genetics Service.

Three women were referred via the Biochemistry Department of the Birmingham Children's Hospital.

B) Staff of the Clinic

All chorionic villus sampling procedures were performed by the author. Ultrasonography was performed either by a Radiographer or the Principal Medical Physicist at the Birmingham Maternity Hospital. A State Registered Nurse and qualified Health Visitor assisted in all procedures, as well as being part of the counselling team for all patients referred. Two Cytogeneticists experienced in the assessment of chorionic villi and in karyotype procedures were present at all samplings to immediately advise the operator on the quality of the villus samples obtained.

C) Transcervical Chorionic Villus Sampling

The preferred method of chorionic villus sampling was transcervical but in certain cases described individually (section 8.4), a transabdominal procedure was used.

The equipment used for each procedure is listed in Appendix 4. An Aluminium cannula described in chapter 4 and illustrated in Figure 3 was used.

The method of sampling was that described in chapter 6, cleansing of the vagina prior to the procedure was performed using 5% Hibitane. Suction was provided by an Electromagnetic Piston Pump (The Reciprator), Figure 19, which could deliver a suction pressure of 70 cms of water maximum (700 mm Hg).

The ultrasound machine used to assess all patients prior to chorionic villus sampling was the General Electric RT 3000 using its 5.0 MHz sector probe. This machine was also used for all transcervical procedures.

D) Transabdominal Chorionic Villus Sampling

Transabdominal chorionic villus sampling was performed using the equipment and method described in chapter 6.

The needle used was an 18 gauge 150 mm spinal needle (Needle Industries, Redditch). 1% lignocaine was infiltrated into the skin (5 mls maximum) in all cases, prior to abdominal puncture.

E) Bacteriological Assessment

The result of a bacteriology swab taken from the endocervix and upper vagina was known no less than 48 hours prior to chorionic villus sampling in all patients. Bacteriology swabs were either sent to the local laboratories by referring Clinicians or if this had not been achieved prior to their assessment at the Birmingham Maternity Hospital, swabs were sent to the Bacteriology laboratory, Birmingham Maternity Hospital. In the laboratory, all swabs were examined for the presence of aerobic and anaerobic organisms as well as fungae. Specific swabs for viral culture were

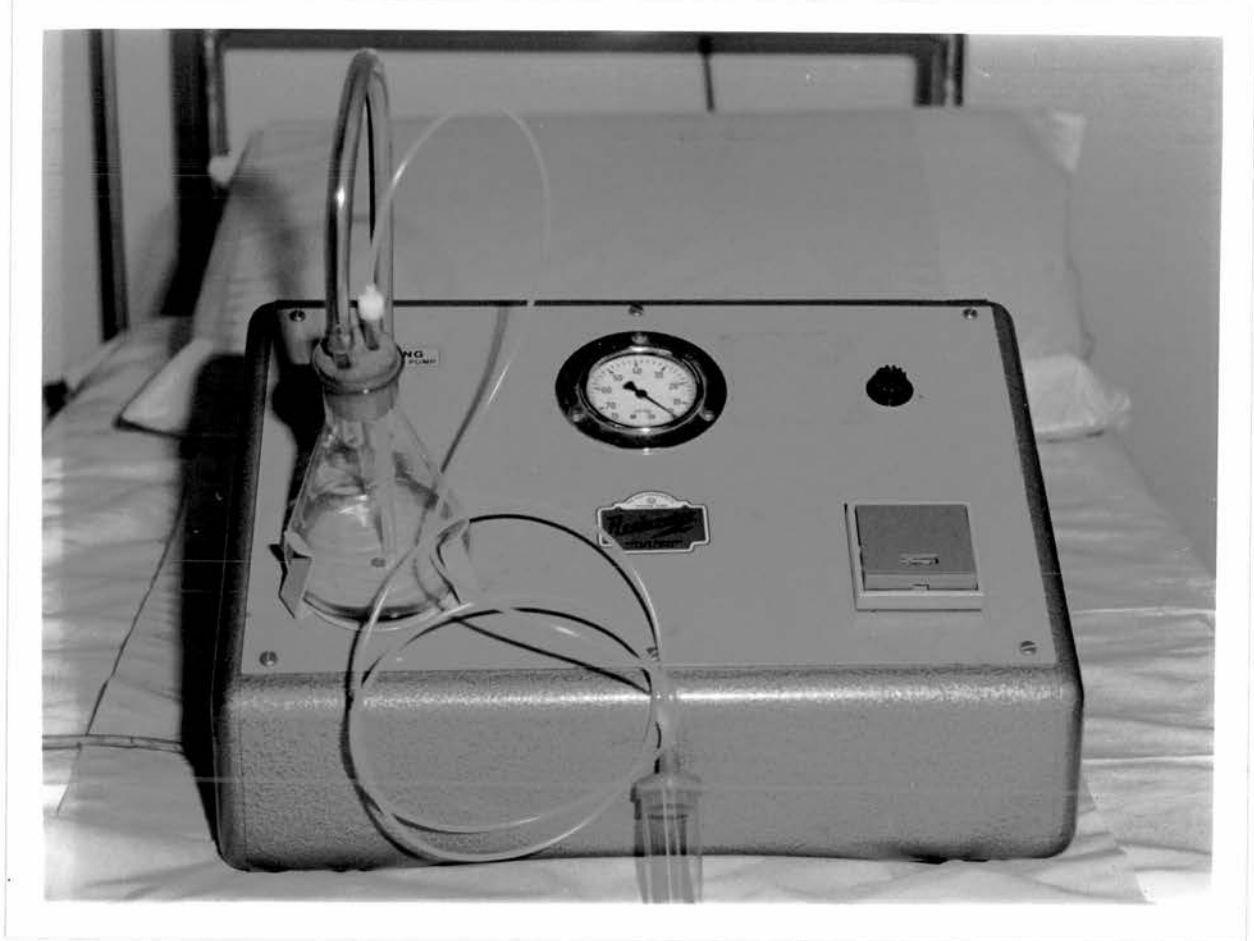


FIGURE 19: RECIPRATOR SUCTION APPARATUS CONNECTED VIA VYGON TUBING TO PAEDIATRIC MUCOUS ASPIRATOR

not requested from any patient, as in none of our cases was a history of herpetic infection obtained. Cultures for Chlamydial organisms are not offered as a routine at the Bacteriology laboratory, Birmingham Maternity Hospital.

In a diagnostic case of transcervical chorionic villus sampling performed in 1984, before this reported study, a septic abortion occurred two weeks after sampling in a patient, in which a preliminary bacteriological report had shown no significant growth. A second report had shown a growth of Bacteroides organisms but by the time specific treatment could be instigated the septic process had commenced. I, therefore, made it a policy that in all women in whom transcervical chorionic villus sampling was performed, irrespective of the preliminary bacteriological report, metronidazole (Flagyl) 200 mgs, three times a day was given after sampling and continued for seven days. This dose of metronidazole in pregnancy has never been proven to be teratogenic in over twenty seven years of clinical use (ABPI, 1984).

Results

Table 33 shows the bacteriological results in the 100 patients.

All patients who had organisms grown prior to chorionic villus sampling had appropriate antimicrobial chemotherapy as indicated on the sensitivity report from the laboratory.

Four of the five patients who had a spontaneous abortion following chorionic villus sampling had no significant growth on bacteriological culture prior to sampling. One patient

TABLE 33

BACTERIOLOGICAL RESULTS

RESULT	Number of Patients
No significant growth	83
Monilia or Candida species	5
Gardnerella Vaginalis	6*
Bacteroides Fragilis	1
Bacteroides Ovalis	1
Beta-Haemolytic Streptococcus	1
Non Beta-Haemolytic Streptococcus	2
Staphylococcus Aureus	1
Total	100

* 1 also had a growth of Peptostreptococcus

had a growth of Gardnerella Vaginalis (patient 71). All 5 had received metronidazole in the appropriate dosage following the procedure. Similarly, in patient 56 who delivered at 26 weeks gestation, there was no growth on bacteriological culture of vaginal swabs prior to her chorionic villus sampling procedure and she also received metronidazole after the procedure.

F) Rhesus Blood Grouping

All patients having chorionic villus sampling had Rhesus blood grouping performed and the result available prior to the procedure.

Any patient found to be Rhesus Negative had a Kleihauer test performed after sampling and an estimation of feto-maternal haemorrhage made.

The standard dose of Anti-D immunoglobulin (250 IU), given to all Rhesus Negative patients after the procedure, was therefore adjusted in line with the Kleihauer report.

Results

Thirteen women were Rhesus Negative and 87 were Rhesus Positive. Of the 13 women who were Rhesus Negative, two (cases 35 and 56) had estimated feto-maternal transfusions of 25 mls and 5 mls respectively by Kleihauer estimation. Patient 35 received a total of 2500 IU of Anti-D and patient 56 received a further 250 IU of Anti-D.

G) Ultrasound Assessment

The gestation of the diagnostic series and the placental location is given in Tables 34 and 35. Gestation was calculated in all cases by measurement of fetal crown rump lengths. Reference tables were modified from Robinson et al 1979.

TABLE 34

GESTATIONAL AGE AT SAMPLING

Gestational age (weeks)	Number
9-10	18
10-11	45
11-12	34
12+	3
Total	100

TABLE 35

PLACENTAL POSITION

	Number	Successful CVS
Anterior	38	38
Posterior	59	57
Fundal	3	2
Total	100	97

8.3 TRANSABDOMINAL CHORIONIC VILLUS SAMPLING

Seven patients had transabdominal chorionic villus sampling performed. The indications for the procedure and the outcome are detailed individually.

Table 36 gives the villus weights for the 7 patients.

Patient Number 22 A 27 year old woman was referred via the Regional Clinical Genetics Service for fetal sexing. She had had 2 previous pregnancies. The first was a spontaneous miscarriage at 6 weeks gestation and the second was a mid-trimester termination of pregnancy at 20 weeks gestation following an amniocentesis. That fetus was male. There was a family history of the Opitz (BBB) syndrome which is sex linked. Males with this condition have several abnormalities including mental retardation. The Clinical Geneticist had estimated a carrier risk of 1 in 4, and a chance of 1 in 8 that a male would be affected (Dr M Hulten, Consultant Clinical Cytogeneticist, East Birmingham Hospital - Personal Communication).

Preliminary high vaginal swab showed no growth. Blood group was A Rhesus Positive. At sampling a single fetus, crown rump length 5.3 cms equivalent to 11½ weeks gestation, was seen. The placental site was anterior. The uterus was in an erect position and anteflexed. Transcervical chorionic villus sampling was attempted twice but satisfactory quantities of villi could not be obtained. Transabdominal chorionic villus sampling was performed and 5-10 milligrams of villi, suitable for analysis, was obtained on one aspiration.

The karyotype was 46,XY.

TABLE 36

VILLUS WEIGHTS

Weight (mg)	Transcervical CVS	Transabdominal CVS
0	7*	0
<10	4	1
10-20	13	4
20-30	15	1
30-40	18	1
40+	40	
Total	97	7 **

* Four subsequently had transabdominal CVS.
Three had amniocentesis.

** Includes the four who had unsuccessful transcervical CVS.

After further counselling by the Regional Clinical Genetics Service, the couple decided to continue with the pregnancy.

The patient had a spontaneous delivery of a live boy, 3.91 kilograms in weight, at 40 weeks + 2 days gestation. There were no neonatal problems and the male child appears to be normal at follow up.

Patient Number 60 A 30 year old woman who had given birth to one male child with primary Trisomy 21. She requested chorionic villus sampling and was referred by her General Practitioner.

Preliminary high vaginal swab showed no growth. Blood group was O Rhesus Positive. At sampling a single fetus, with a gestational age equivalent to 10-11 weeks, was seen. The placental site was posterior and fundal. Two attempts at transcervical chorionic villus sampling were unsuccessful as the bulk of the placenta was in a fundal and inaccessible position. She was given metronidazole, 200 mgs twice daily, for 7 days, and 5 days later at 11 weeks gestation underwent transabdominal sampling when 10 milligrams of villi were obtained.

The fetal karyotype was 46,XX.

She had a spontaneous delivery at 40 weeks + 1 day gestation, of a live female, 3.2 kilograms in weight. There were no neonatal problems.

Patient Number 77 This 39 year old woman was referred by her Obstetrician for prenatal screening. She entered the MRC trial and was allocated chorionic villus sampling.

Preliminary high vaginal swab showed Gardnerella Vaginalis and she was treated with metronidazole, 200 mgs three times a day, for 7 days, starting on the day of sampling. She was Rhesus Positive. On ultrasonography, the fetus had a crown rump length of 3.9 cms equivalent to 10-11 weeks gestation. A cystic fibroid was noted posteriorly on the uterine wall distorting the endocervical canal.

Consequently, she had transabdominal chorionic villus sampling and 15 milligrams of villi were obtained with one aspiration.

The fetal karyotype was 46,XX.

She had a Caesarean Section at 37 weeks and 4 days gestation. A live female, 3.15 kilograms in weight, was delivered. There were no neonatal complications.

Patient Number 80 This 36 year old woman was referred for prenatal screening because of her age. She was entered into the MRC trial and was allocated chorionic villus sampling.

Preliminary high vaginal swab showed no growth. She was A Rhesus Positive. At sampling, ultrasonography revealed a single fetus with a gestational age of 11 weeks. There was a large cervical fibroid, 5 x 6 cms, distorting the endocervical canal. Transabdominal chorionic villus sampling was performed and 20 milligrams of villi were obtained on one aspiration.

The fetal karyotype was 46,XX.

She had a normal delivery at 40 weeks + 3 days gestation of a live female, 2.7 kilograms in weight. There were no neonatal complications.

Patient Number 87 This 36 year old woman who had had a previous Down's fetus terminated at twenty weeks gestation, was referred by her Obstetrician for chorionic villus sampling. She had had one other normal pregnancy and delivery.

Preliminary high vaginal swab showed no growth. She was O Rhesus Positive. At sampling, ultrasonography revealed a fundal and anteriorly placed placenta. A single fetus with a gestational age of 10 weeks was seen. The uterus was anteflexed and the placental site could not be reached by the transcervical route. One attempt was made. Trans-abdominal chorionic villus sampling was performed and 10 milligrams of villi were obtained.

The fetal karyotype was 46,XY.

She had a spontaneous delivery at 39 weeks + 3 days gestation of a live male, 3.8 kilograms in weight. There were no neonatal complications.

Patient Number 93 This 37 year old woman was referred by her General Practitioner for prenatal diagnosis because of age. She entered the MRC trial and was allocated chorionic villus sampling.

Preliminary high vaginal swab was negative. She was A Rhesus Negative. At sampling a single fetus, with a gestational age equivalent to 10 weeks, was seen. The placental site was anterior. Transcervical chorionic villus

sampling proved impossible because her endocervical canal was narrow and rigid, and the anteriorly placed placenta could not be reached. Transabdominal chorionic villus sampling was performed and 35 milligrams of villi were obtained following two aspirations.

The fetal karyotype was 46,XX.

A Kleihauer test performed after the procedure showed no fetal cells. 250 IU of Anti-D were given after the procedure.

She had a spontaneous delivery at 41 weeks gestation of a live female, 3.5 kilograms in weight. There were no neonatal complications.

Patient Number 98 This 23 year old woman was referred by the Regional Clinical Genetics Service. She had had one pregnancy resulting in a boy suffering from Cystic Fibrosis. The family were fully informative with DNA cystic fibrosis gene probes.

Preliminary high vaginal swab was negative. She was O Rhesus Positive. At sampling, ultrasonography showed a single fetus, gestational age 10-11 weeks. Placental site was posterior and fundal. The mainly fundal placental site could not be entered satisfactorily via the trans-cervical route. Transabdominal chorionic villus sampling was performed and approximately 30 milligrams of villi were obtained. Metronidazole in the standard dose was given for 7 days.

The results of the DNA probing revealed that the fetus was neither a carrier nor affected with Cystic Fibrosis. Cytogenetic analysis of 32 metaphases showed 24 cells with 46 chromosomes (46,XY) and 8 cells with 47 chromosomes (47,XY,+21) indicating chromosomal mosaicism.

After counselling by the referring Clinical Geneticists, the patient opted for a termination of pregnancy and this was performed at 13 weeks gestation. Chromosomal mosaicism will be discussed in section 8.6 B.

8.4 TRANSCERVICAL CHORIONIC VILLUS SAMPLING

Ninety three patients had transcervical chorionic villus sampling performed using the method described in chapter 6.

In three of these patients, insufficient villi were obtained for diagnostic use and all 3 subsequently had an amniocentesis (patients 5, 57 and 74).

Ninety patients had successful transcervical chorionic villus sampling and the villus weights are recorded in Table 36.

A) Failed Transcervical Chorionic Villus Sampling

Patient Number 5 A 41 year old woman who had had 3 normal children, was referred for chorionic villus sampling by her Obstetrician because of her age.

Preliminary high vaginal swab was negative. Blood group was B Rhesus Positive. At sampling, a single fetus with a gestational age of 10 weeks was seen. Placental site was fundal. The cannula was inserted in an anterior direction twice and a posterior direction once but no villi could be obtained. After counselling, she chose amniocentesis rather than repeat chorionic villus sampling.

The patient had a normal delivery of a live male, 3.71 kilograms in weight, at term. There were no neonatal problems.

Patient Number 57 A 26 year old woman who had given birth to a child with Trisomy 21, was referred by the Clinical Genetics Service, for chorionic villus sampling.

Preliminary high vaginal swab was negative. Blood group was O Rhesus Positive. At sampling, a single fetus equivalent to 10 weeks gestation was seen. The placental site was posterior and fundal. The uterus was retroverted and fixed. The placental site could not be reached by the transcervical route and transabdominal chorionic villus sampling was thought to be impossible without breaching the amniotic cavity. She was seen one week later, at 11 weeks gestation, but the position had not altered and an amniocentesis was recommended. This procedure was carried out at 17 weeks and the fetal karyotype was normal.

She had a normal delivery at 40 weeks gestation of a live female, 3.58 kilograms in weight. No neonatal problems were encountered.

Patient Number 74 A 39 year old woman was allocated chorionic villus sampling after entering the MRC randomised trial.

At sampling a single fetus, equivalent to 11 weeks gestation, was seen. Placental site was posterior. Two aspirations were attempted. There was poor visualisation of the cannula due to her obesity. Brisk bleeding of about 20 millilitres occurred at the second aspiration. The procedure was stopped. No villi were obtained.

Her bleeding continued off and on for a further ten days and she was admitted to hospital. Her pregnancy continued and she had an uneventful amniocentesis at 17 weeks gestation.

She had a spontaneous rupture of membranes at thirty-four weeks gestation and delivered a live male, 2.42 kilograms in weight. There were no neonatal problems.

B) Comment

Patient number 5 may have benefitted from a trans-abdominal approach initially rather than a transcervical and I believe, in those cases with fundal placentae this should, and now is, the desired approach.

Patient number 57, with a retroverted uterus and a postero-fundal placenta, is an example of a patient in whom chorionic villus sampling in the first trimester is not possible. Second trimester villus sampling may well be easier in such cases.

Despite a heavy loss of blood, the pregnancy was not compromised in patient number 74. Whether her chorionic villus sampling or amniocentesis was responsible for her premature rupture of membranes is difficult to say. There was no antenatal or post natal evidence of infection, as a cause for ruptured membranes in her case.

8.5 INDICATIONS FOR CHORIONIC VILLUS SAMPLING

A) Maternal Age

The general policy of the West Midlands Regional Cytogenetics Service is to offer prenatal testing for chromosomal abnormalities to women aged 36 years and over at the start of their pregnancies (from October 1987, this changed to 35 years and over). The risk factors for Down's Syndrome quoted to such women is shown in Table 37 and is derived from data from the European Collaborative Study of Amniocentesis 1984 (Ferguson-Smith 1984).

All patients wishing chorionic villus sampling where the risk was maternal age entered the Medical Research Council trial into chorionic villus sampling and amniocentesis, and after due counselling were randomised to receive either chorionic villus sampling or amniocentesis.

A total of 42 patients were assigned to chorionic villus sampling. The ages of the referred patients are shown in Table 38. Three had transabdominal chorionic villus sampling (section 8.3). Two patients had unsuccessful transcervical chorionic villus sampling performed (section 8.4 A). The remaining 37 patients had successful transcervical procedures. Table 39 shows the karyotype results.

The outcome of the 42 patients who had successful or unsuccessful chorionic villus sampling is shown in Table 40. The extrapolated spontaneous abortion rate for this group is 7.5%.

TABLE 37

Age	Risk of Down's Syndrome
25	1 in 1,500
30	1 in 800
35	1 in 350
36	1 in 300
37	1 in 200
38	1 in 170
39	1 in 140
40	1 in 100
45	1 in 30

TABLE 38

MATERNAL AGE: AGE RANGES OF THE 42 PATIENTS

Patient age	Number
36	4
37	10
38	7
39	12
40	2
41	4
42	2
43	1

TABLE 39

MATERNAL AGE: SUCCESSFUL CVS WITH KARYOTYPES

Karyotype	Number
46,XX	14
46,XY	20
*46,XY/47,XY,+15	1
*46,XY/47,XY,+3	1
*46,XX/45,X	1

* These cases are discussed in section 8.6 B.

TABLE 40

OUTCOME OF PREGNANCY IN 42 PATIENTS HAVING CHORIONIC VILLUS SAMPLING FOR MATERNAL AGE

	Number
Delivered live baby > 37 weeks gestation	32
Delivered live baby < 37 weeks gestation	4
Stillbirth	1
Spontaneous abortion	3
Termination of pregnancy	2

Stillbirth Patient Number 72 This 38 year old woman had transcervical chorionic villus sampling at 11 weeks gestation. Preliminary high vaginal swab showed no growth. She was Rhesus Positive. The placental site was posterior. Twenty five milligrams of villi were obtained on one aspiration.

The fetal karyotype was 46,XY.

There were no ante natal problems until thirty three weeks gestation when she was admitted with severe pre-eclampsia complicated by disseminated intra vascular coagulation and renal failure. On admission to the labour ward, the fetus was found to be dead.

Post mortem of the fetus and placenta was performed. The male fetus weighed 1.93 kilograms (between 10th and 50th centile for gestational age and sex). There were no external or internal congenital malformations. The histology of the placenta showed acute ischaemic changes and massive perivillous fibrin deposition. The umbilical cord and membranes were normal.

The cause of fetal death was due to acute intrauterine hypoxia secondary to her pre-eclampsia.

B) Previous Chromosomal Abnormality

Twenty six patients who had had a fetus or child with a previous chromosomal abnormality were referred for chorionic villus sampling. Table 41 details the previous abnormalities.

One patient had unsuccessful chorionic villus sampling (patient 57, section 8.4 A).

The karyotypes diagnosed by chorionic villus sampling of the remaining 25 is shown in Table 42.

TABLE 41

CVS IN PATIENTS WITH PREVIOUS CHROMOSOMAL ABNORMALITY

PREVIOUS ABNORMALITY	NUMBER
Trisomy 21	22
Trisomy 18	2
45,X	1
Wolf-Hirschorn Syndrome (46,XX,r(4)(p16q35)	1
Total	26

TABLE 42

KARYOTYPES IN 25 SUCCESSFUL CVS PROCEDURES FOR PREVIOUS CHROMOSOMAL ABNORMALITY

PREVIOUS ABNORMALITY	KARYOTYPE
Trisomy 21 (n=21)	46,XY (n=12) 46,XX (n=7) 46,XY/47,XY,+3 (n=2)*
Trisomy 18 (n=2)	46,XX (n=1) 46,XY (n=1)
45,X (n=1)	46,XY (n=1)
Wolf-Hirschorn Syndrome (n=1)	46,XY (n=1)

* Discussed in section 8.6 B.

Discussion

Twenty six of the 100 diagnostic procedures were performed in patients with previous chromosomally abnormal fetuses or children.

Prior chromosomal abnormality was the indication for chorionic villus sampling in 8.4% of a Dutch series of 500 patients (Leschot et al 1987), 4.6% of a 1000 diagnostic chorionic villus sampling patients from California (Hogge et al 1986) and 3.6% of 109 chorionic villus sampling patients reported from Chicago by Elias et al 1986. In a further 63 patients who have had diagnostic chorionic villus sampling in our unit in Birmingham, a quarter were for a previous chromosomal abnormality, continuing the pattern of the previous 100 patients.

Although in the twenty six patients there was one fetal loss (patient 27, section 8.8), it may be that in diagnostic series in which previous aneuploidy forms a significant proportion of the total, spontaneous miscarriage may be more common.

Series such as ours may then be at risk of showing an excess number of miscarriages in comparison with other series in which prior chromosomal abnormality forms a small proportion of patients having chorionic villus sampling. Such factors make comparisons of spontaneous fetal losses between centres offering chorionic villus sampling, difficult (Simpson et al 1986).

Two patients (39 and 100) had mosaicism and this will be discussed in detail in section 8.6 B.

C) Chorionic Villus Sampling For Fetal Sexing

Fourteen chorionic villus sampling procedures for fetal sexing were carried out, where the indication was a family history of an X-linked condition.

One patient (8 and 28) had chorionic villus sampling carried out in successive pregnancies.

All patients were referred from the Regional Clinical Genetics Service.

Indications

(i) Family History of Duchenne Muscular Dystrophy

Table 43 details the outcome of 10 procedures for this indication. One patient (who had 2 chorionic villus sampling procedures) will be used to illustrate the problems with chorionic villus sampling in patients with this condition.

Ten procedures were performed in nine patients.

Patient Number 8 and 28 A 23 year old woman who had two brothers who both died from Duchenne Muscular Dystrophy was referred for chorionic villus sampling. In her first pregnancy, she had an amniocentesis showing a female karyotype and subsequent normal delivery. In this pregnancy, she had chorionic villus sampling between 9 and 10 weeks gestation by dates and ultrasound assessment.

High vaginal bacteriology swab was negative. Blood group was O Rhesus Positive. The placental site was posterior.

Fifty milligrams of villi were obtained on two aspirations.

TABLE 43

CHORIONIC VILLUS SAMPLING FOR FETAL SEXING IN CASES WITH A FAMILY HISTORY OF DUCHENNE MUSCULAR DYSTROPHY

Patient number	Gestation (weeks)	Villus weights (mgs)	Karyotype	Outcome (birth details, gestation, weight)
8	9+	50	46,XY	T.O.P.
& 28	11	30	46,XY	T.O.P.
23	9+	40	46,XX	Live female, 40+weeks, 3.97 kgs.
25	11	30	46,XY	T.O.P.
29	10	5	46,XX	Live female, 41 weeks, 3.67 kgs.
32	10+	30	46,XX	Live female, 42 weeks, 3.82 kgs.
50	9+	40	46,XX	Live female, 39 weeks, 2.82 kgs.
64	10	30	46,XX	Live female, 39 weeks, 2.3 kgs.
78	10+	60	46,XX	Live female, 41 weeks, 3.9 kgs.
81	10+	40	46,XY	T.O.P.

T.O.P. Termination of pregnancy

kgs Kilograms

The fetal karyotype was 46,XY.

She had a termination of pregnancy performed at 11½ weeks gestation.

Six months later, she presented in her third pregnancy. At sampling, a single fetus, crown rump length 4.6 cms, equivalent to 11 weeks gestation was seen.

High vaginal bacteriology swab was negative. Placental site was anterior.

Thirty milligrams of villi were obtained on one aspiration.

The fetal karyotype was 46,XY.

She underwent termination of pregnancy at 13 weeks gestation.

She presented in her fourth pregnancy for chorionic villus sampling. By her last menstrual period, she should have been eight weeks gestation, but on ultrasound scan, two sacs were seen with no embryo in either. She subsequently underwent evacuation of the uterus at her local hospital.

Discussion

At the time these patients had chorionic villus sampling for fetal sexing (1986 and 1987), accurately pinpointing affected male fetuses using DNA analysis was not possible. In the illustrative case, the affected brothers were both dead and therefore DNA analysis of them was impossible. This, therefore, left fetal sexing as the only option, even though

normal males may be terminated. A succession of pregnancies in which males are diagnosed, as in this case, often leads to much despair and eventually voluntary infertility on behalf of the couple concerned.

Until such time that specific DNA probes that avoid recombination at the Duchenne Muscular Dystrophy locus are available, accurate prenatal diagnosis will be difficult. In those families without informative relatives, accurate diagnosis of affected males will be difficult, although linkage analysis may be possible (Forrest et al 1987).

(ii) Family History of Becker Muscular Dystrophy Patient Number 88 This patient, who had a living brother with Becker Muscular Dystrophy, was referred for chorionic villus sampling. Prior to becoming pregnant, she had sought advice from the Clinical Genetics Department about her chances of being a carrier for this condition and whether prenatal diagnosis was possible. Her brother had been shown on DNA studies (Dr Kay Davies, Oxford), to have a deletion of his Becker gene. At the time of referral, this DNA probe had only been in use for a few weeks.

DNA results on the patient suggested that the patient was not a carrier and her overall risk was put at 1 in 60, with a 1 in 120 chance of having an affected son (Dr S Bundey, Clinical Genetics Department, Birmingham Maternity Hospital). Nevertheless the couple were keen on prenatal diagnosis, even at such low risk.

High vaginal bacteriology swab was negative. Blood group was O Rhesus Positive. At sampling, a single fetus,

crown rump length 3.9 cms was seen, equivalent to 10+ weeks gestation. Placental site was posterior.

Thirty milligrams of villi were obtained on one aspiration, sufficient for karyotype analysis and DNA analysis.

The fetal karyotype was 46,XX and therefore further investigation was not required.

She had a spontaneous delivery at 40 weeks gestation of a live female, 3.64 kilograms in weight. There were no neonatal complications.

(iii) Family History of Opitz (BBB) Syndrome Patient Number 22 is detailed in section 8.3.

(iv) Family History of Fragile X Syndrome Patient Number 47 This 25 year old woman had one male child who was mentally retarded and hyperactive. He had been diagnosed as having the Fragile X Syndrome. When this case presented, the most reliable method of prenatal diagnosis was by the cytogenetic analysis of the fetal lymphocytes obtained by fetal blood sampling (Webb et al 1987).

In this case, the chorionic villus sampling diagnosis of a male karyotype would be followed by later fetal blood sampling.

Preliminary high vaginal swab yielded some yeasts. Canestan pessaries, one at night for three consecutive nights, were prescribed before sampling. Blood group was O Rhesus Positive. At sampling, ultrasonography revealed a single

fetus, crown rump length 3.5 cms, equivalent to 10 weeks gestation. Placental site was posterior.

Sixty milligrams of villi were obtained on one aspiration.

The fetal karyotype was 46,XX.

She had an elective Caesarean Section at 40 weeks gestation. A live female, 3.64 kilograms in weight, was delivered. There were no neonatal problems.

(v) Family History of XY Gonadal Dysgenesis

Patient Number 99 This 21 year old woman had an extensive family history of XY Gonadal Dysgenesis, which is inherited in an X-linked manner. Her mother had an affected female child (i.e. one in which a female phenotype was apparent but with an XY karyotype and dysgenetic gonads).

Patient number 99 had already had a phenotypically normal male child. She was counselled and referred via the Regional Clinical Genetics Service with the understanding that if chorionic villus sampling diagnosed an XY karyotype, then high resolution ultrasound or fetoscopy would then be necessary to confirm the presence of male genitalia.

Preliminary high vaginal swab was negative. Blood group was A Rhesus Positive. At sampling, a single fetus, crown rump length 3.5 cms was seen, equivalent to 10 weeks gestation. Placental site was anterior.

Forty milligrams of villi were obtained on two aspirations.

The fetal karyotype was 46,XY.

At 18+ weeks gestation, she had ultrasonography performed and male genitalia were seen.

She had a normal delivery of a live, anatomically normal, male at 41 weeks and 4 days gestation. Birth weight was 3.8 kilograms. There were no neonatal complications.

D) Fetal Karyotyping Because of a Familial Translocation

Patient Number 41 A 32 year old woman, whose husband had a balanced translocation 46,XYt(4;15)(q25;q26) was referred for chorionic villus sampling. They had had one male child who died and was found to have the unbalanced form of his father's condition.

Preliminary high vaginal swab showed no significant growth. Blood group was O Rhesus Positive. At sampling, a single fetus with a gestational age of 9-10 weeks was seen. The placental site was anterior. Two aspirations produced 25 milligrams of villi.

The karyotype result was 46,XY,-15,+der,t(4;15)(q25;q26)pat.

Termination of pregnancy was performed at 12 weeks gestation and the products of conception sent to the laboratory for chromosomal analysis. The result was 46,XY,-15,+der(15),t(4;15)(q25;q26)pat. and confirmed the chorionic villus sampling result.

Patient Number 49 A 35 year old woman, a known carrier of the 14;21 translocation was referred by the Clinical Genetics Service for chorionic villus sampling. Her karyotype was

45,XX,t(14q21q). She had had one termination of pregnancy for social reasons, one healthy chromosomally normal female and a girl affected with Down's Syndrome. She had been given a risk of 1:7 of any future pregnancy being affected with Down's Syndrome (Dr M Hulten, Consultant Clinical Cytogeneticist).

Preliminary high vaginal swab showed no significant growth. Blood group was A Rhesus Positive. A single fetus, gestational age 10 weeks, was seen. Placental site was posterior. Between 10 and 20 milligrams of villi were obtained on two aspirations.

In 13 of 15 cells analysed, the karyotype was 46,XY,-14, +t(14q21q), consistent with a clinical picture of Down's Syndrome.

Termination of pregnancy was performed at 12 weeks gestation and products of conception sent to the laboratory for analysis. Culture of these products showed a karyotype 46,XY,-14,+t(14q21q), thus confirming the result of the chorionic villus sampling procedure.

This patient was subsequently sterilised at her own request.

Patient Number 90 A 28 year old woman, whose husband had a Robertsonian translocation was referred by her General Practitioner. She had had an amniocentesis in her one previous pregnancy and had a normal delivery of a live female with a normal karyotype.

Preliminary high vaginal swab showed no growth. Blood group was O Rhesus Positive. At sampling, two sacs were

seen, one empty and one with a fetus with a crown rump length of 4-6 cms equivalent to 11-12 weeks gestation. The placental site was posterior and 15 milligrams of villi were obtained in one aspiration.

The fetal karyotype was 46,XY.

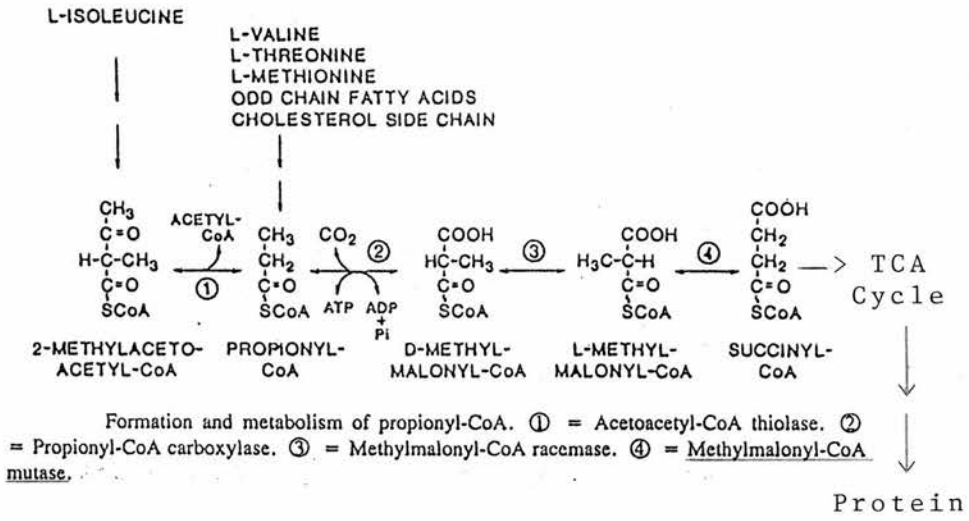
At 37 weeks gestation, she had a spontaneous delivery of a live male, 3.57 kilograms in weight. There were no neonatal complications.

E) Biochemical Analysis

Three patients, whose cases will be described individually were referred for chorionic villus sampling by the Regional Clinical Genetics Department and the Biochemistry Department, Birmingham Children's Hospital.

Patient Number 6 This 31 year old woman had given birth to a child in 1985, who died in the early neonatal period with methylmalonic aciduria. This is an autosomal recessive condition with a one in four recurrence risk. The condition is not amenable to dietary treatment after birth.

She became pregnant in 1986, and was seen by a Consultant Clinical Geneticist. The enzyme defect in the family had been shown to be a Methylmalonyl-CoA mutase deficiency. This enzyme acts in the formation of protein as shown in the following diagram.



Formation and metabolism of propionyl-CoA. ① = Acetoacetyl-CoA thiolase. ② = Propionyl-CoA carboxylase. ③ = Methylmalonyl-CoA racemase. ④ = Methylmalonyl-CoA mutase.

By incorporating radiolabelled carbon (Carbon 14) as propionate into the sample, its detection in synthesised protein would mean there is no block to its incorporation in the pathway outlined above and therefore its presence indicates an unaffected individual.

The couple were referred for chorionic villus sampling, and understood that if the biochemical result from the chorionic villus sample was normal, an amniocentesis, for confirmation would be necessary. The arrangements for biochemical analysis of the villi were that the sample would be sent to Dr W Kleijer's laboratory in Rotterdam, who had previously monitored four pregnancies with this condition using chorionic villus samples (3 unaffected and one affected which was terminated).

A high vaginal swab taken prior to sampling grew Candida species and a Beta haemolytic streptococcus, treated successfully with Canestan pessaries and oral Erythromycin. Blood group was A Rhesus Negative. At sampling, a single

fetus, crown rump length 3.1 cms was seen. Placental site was posterior.

Ten milligrams of villi were obtained on two aspirations. A Kleihauer test was done after the procedure and this was negative. Anti-D, 250 IU was given. There were no immediate problems.

RESULT:- The Rotterdam laboratory showed that C^{14} propionate incorporation was substantial in the villus sample consistent with a fetus unaffected with methylmalonic aciduria.

The couple were told this result with the caveat that amniocentesis for confirmation would be necessary.

Amniocentesis was performed at 17½ weeks gestation.

RESULT

AMNIOTIC FLUID SUPERNATANT

Methyl citrate micromoles L^{-1}	
Fetus	8.16
Positive Control	7.36
Negative Control	1.56
Normal Range (±2 SD)	0.18 - 0.58
Methylmalonate micromoles L^{-1}	
Fetus	42.2
Positive Control	30.3
Negative Control	0.26, 0.08, 0.15, not detected.
Normal Range (±2 SD)	0-0.8

Affected values more than 13.

CULTURED AMNIOTIC FLUID CELLS

	moles propionate/mole phenylanine incorporated x 10^{-3}
Fetus (Triplicate of separate cultures)	7.9, 4.7, 7.1
Controls (6)	98.0 (± 26.8 (Mean ± SD)) (71.1 - 136 (Range))

CULTURED SKIN FIBROBLASTS

Previous affected child	6.7
Normal Control	88.2

These results showed that the fetus was affected with methylmalonic aciduria.

After counselling from the Consultant Clinical Geneticist, the patient underwent extra amniotic Prostaglandin termination of pregnancy at 20+ weeks gestation.

Cultured fibroblasts from the terminated fetus were consistent with the amniocentesis result, demonstrating a fetus affected with methylmalonic aciduria.

Patient Number 19 A 17 year old girl presented to the Clinical Genetics Department, approximately eight weeks pregnant. Her sister (patient number 75) had had a son who had died with Menkes' Disease.

Menkes' Disease is a syndrome of congenital copper disturbance characterised by increased copper accumulation in multiple cell types in the body. Affected male fetuses accumulate excessive amounts of copper in placental tissue and this accumulation is said to be useful in prenatal diagnosis, with the principal site of copper accumulation being the trophoblast (Horn 1981).

In 1985, an affected male fetus had been diagnosed using chorionic villus sampling in the tenth week of pregnancy and the diagnosis verified after abortion (Tønnesen 1985).

Unfortunately carrier detection tests for Menkes' Disease had not been carried out on patient number 19 as this pregnancy was unexpected.

Her sister, a known Menkes' Disease carrier (patient number 75) had had chorionic villus sampling successfully performed in 1985, and patient number 19 wished to avail herself of this test. She was given a risk of a male fetus being affected by Menkes' Disease of 1 in 4 by the Clinical Geneticists, and because experience worldwide with this test was small a confirmatory amniocentesis was recommended.

Prior to sampling, an endocervical bacteriology swab had shown no significant growth. Blood group was O Rhesus Positive. At sampling, a single fetus, crown rump length 4.7 cms was seen, equivalent to 11 weeks gestation. Placental site was anterior.

Two aspirations were made using a Portex catheter (Portex Limited), as described in chapter 3, but with the suction device, as described in chapter 6 attached. The choice of this catheter will be explained in describing patient number 75. Ten milligrams of villi were obtained and the villi were cleaned and packed in a copper free solution to Dr Tønnesen's laboratory in Denmark. A further sample, approximately 30 milligrams was obtained using an aluminium cannula for fetal karyotyping.

The fetal karyotype was 46,XY.

BIOCHEMICAL RESULT

Copper Content	0.66	parts per million
Control	1.0	parts per million
Normal Male	0.4-1.1	parts per million

These results suggested a fetus unaffected with Menkes' Disease.

She had amniocentesis performed at 16 weeks gestation.

RESULT

CULTURED AMNIOTIC FLUID CELLS

			ng^{64} Cu. $20hr^{-1}mg^{-1}$protein
Patient	Culture 1		16.1
	Culture 2		12.7
Control (1)		run in	11.2
Menkes'(Fibroblasts)(1)		parallel	97.5

Reference Ranges	Mean	95% limits of range
Menkes' amniotic fluid cells(12)	53.7	37.0-95.0
Control amniotic fluid cells(20)	17.7	7.8-27.8

These results were not consistent with a diagnosis of Menkes' Disease.

These results agreed with the villus results. At 38+ weeks gestation, she had a normal delivery of a live male, 2.87 kilograms in weight.

Serum copper and caeruloplasmin from the child confirmed that it was unaffected with Menkes' Disease.

Patient Number 75 This 24 year old woman, the elder sister of patient number 19, had had two children. A healthy boy born in 1982 and a boy in 1983 who died at the age of 7 months from Menkes' Disease. She was referred via the Biochemistry Department, Birmingham Children's Hospital for prenatal diagnosis.

She had chorionic villus sampling performed in our unit in 1985 and 1987 in her third and fourth pregnancies.

Third Pregnancy - 1985

Preliminary high vaginal swab was negative. Blood group was O Rhesus Positive. At sampling, a single fetus, 9 weeks gestation was seen. The placental site was posterior.

Transcervical chorionic villus sampling was performed using the method described in chapter 3. Sufficient villi for biochemical analysis was obtained. Two samples from women undergoing first trimester termination of pregnancy were sent as controls. These had been taken using a malleable stainless steel catheter (chapter 3).

BIOCHEMICAL RESULT

		Nanograms copper/ milligrams wet weight
Patient	Aluminium Cannula	3.50
Control 1	MSS Cannula	2.42
Control 2	MSS Cannula	2.85
Normal Controls (n=12)		0.24-1.02
Diagnostic sample (n=9)		0.20-0.80

These results from Dr T Tønnesen, John F Kennedy Institute, Glostrup, Denmark, were difficult to interpret. Their previous experience had all been with villus samples obtained using the Portex cannula (Portex Limited) which had a plastic cannula (though inner aluminium obturator which was removed after placement but prior to villus sampling).

The patient underwent amniocentesis at 16 weeks gestation.

The fetal karyotype was 46,XX.

AMNIOTIC FLUID CELLS

		ng Cu^{64} uptake/mg protein/20h
Patient	Culture A	24.8
	Culture B	20.3
Controls (n=2)		15.0; 17.4
Menkes' Fibroblasts (n=1)		86.1

Reference Ranges

Menkes' amniotic fluid cells (n=12)	37.0-95.0
Control amniotic fluid cells (n=90)	7.8-27.8

These results were consistent with a fetus unaffected with Menkes' Disease.

The discrepancy in the copper uptake values in the controls and in the patient's villus samples which had been taken with metal cannulae was possibly due to copper contamination from the cannulae themselves. However, an enquiry to the manufacturers of the metal cannulae (Rocket of London Limited), had shown that the aluminium cannula

was made from commercial grade material which was 96% pure aluminium and the remainder was made up of various elements, including copper, to produce the various quantities of the alloy. The stainless steel tubing was allowed a maximum of 0.1% of copper and ideally should contain none at all. Heavier concentrations of copper were found in the brass Luer fittings, which contained 2/3 copper. The jointing metal was called silver solder, and this had a 30% copper content. The jointing material on the aluminium cannula was either cyanoacrylate or epoxy resin. Therefore the manufacturers would expect very small quantities of the hub to be exposed to the incoming sample. The male Luer fitting on the collecting syringe would take up most of the available space, and the tubing was deeply counter bored into the fitting.

At forty weeks gestation, the patient had a normal delivery of a live female, 4.1 kilograms in weight.

Samples of the placenta and umbilical cord were sent to Denmark for analysis. Measurement of placental copper was normal but measurement of radioactive copper uptake (Cu^{64}) in the umbilical cord showed the child to be a carrier.

Fourth Pregnancy - 1987

In her fourth pregnancy, the patient requested chorionic villus sampling. Because of the problems with interpretation of the copper content on villus samples taken with the aluminium cannula in her third pregnancy, it was decided to use the Portex Cannula for sampling.

Transcervical chorionic villus sampling was performed at 9-10 weeks gestation. A single fetus was seen and the placental site was posterior. High vaginal swab was negative. Forty milligrams of villi were obtained.

The fetal karyotype was 46,XY.

BIOCHEMICAL RESULT

CHORIONIC VILLUS SAMPLES (Dr Tønnesen, Denmark)

	Copper mg Kg^{-1}
Patient	6.3
Control 1	2.27
Control 2	0.63
Control Range (n=26)	0.24-1.30

The conclusions from Dr Tønnesen's laboratory was that the male fetus was affected with Menkes' Disease. Previous experience (4 affected fetuses) had shown fetuses with these ranges of results to be affected.

After counselling with the patient's Obstetrician and the Clinical Genetics Department, she had a termination of pregnancy performed at twelve weeks gestation. Samples of fetal tissue were sent to the Danish laboratory for confirmation of the diagnosis.

RESULT

CHORIONIC VILLUS BIOPSY
(Pre-termination)

	mg Kg^{-1} Copper
Fetus	5.1
Normal Control	0.24-1.30

CULTURED FETAL FIBROBLASTS

	ng Cu^{64} incorporated $20hr^{-1} mg^{-1}$ protein
Fetus (Male)	67.2
Control (in parallel)	22.3
Menkes' Syndrome (in parallel)	77.8
Normal Male Controls (20) 95% range	9.0-33.3
Menkes' Syndrome (26) 95% range	46.6-99.9

These results were consistent with a diagnosis of Menkes' Disease and confirmed the result from the chorionic villus sample.

DISCUSSION

The discrepancy between the result obtained from the chorionic villus sample and the amniotic fluid sample was of great concern to all those involved in the management of case number 6. The specific enzyme Methylmalonyl-CoA mutase could not be used as a direct measurement in the chorionic villus sample in this particular family as the specific defect was in a co-enzyme essential to the function of the mutase and the mutase levels themselves were normal in the child that had died. One of the reasons put forward by the Dutch laboratory for the incorrect diagnosis was maternal cell contamination of the chorionic villus sample. This is possible, but the sample was sent 'cleaned' to the laboratory and a further washing and cleaning of the sample took place in the Dutch laboratory. Another possibility is that the piece of villi sent was 'dead', and therefore no enzyme activity was present, but this would be likely to give such

abnormal results that suspicions would be raised (G Gray, Principal Biochemist, Birmingham Children's Hospital- Personal Communication).

Despite much discussion between the Dutch laboratory and the laboratory in Birmingham, no satisfactory explanation was given as to why the villus result was incorrect. This led to chorionic villus sampling not being recommended to this couple in their next pregnancy and the patient had an amniocentesis, with subsequent normal results.

In the two cases of Menkes' Disease, it was discovered that copper contamination from the sampling instruments could lead to confusing results (though not a true false positive). The laboratory concerned (in Denmark) now make it a policy that chorionic villi sent to them for copper measurements be taken using an all plastic cannula (Tønnesen et al 1987).

These cases illustrate the difficulty of using chorionic villi for biochemical analysis in cases of certain inborn errors of metabolism. Proper diagnostic assessment should include biochemical analysis of the fibroblasts of an index case and confirmation of any negative result from a villus sample by amniotic fluid analysis.

F) DNA Analysis from Chorionic Villi

Twelve patients were referred for chorionic villus sampling, for DNA analysis.

Indications

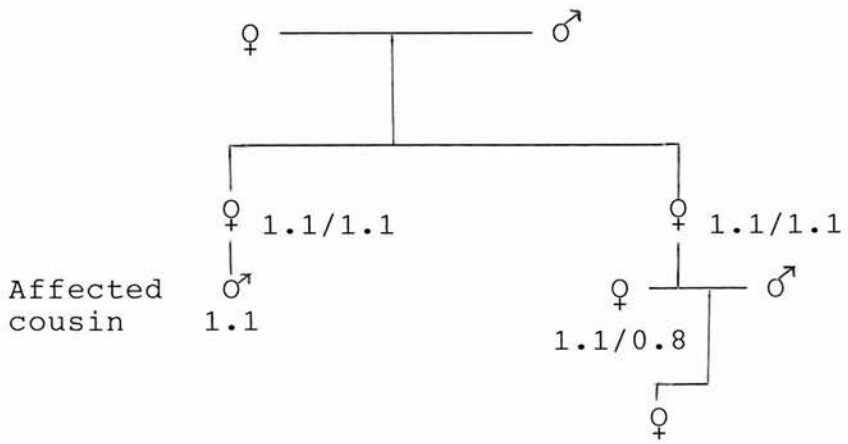
(i) Haemophilia A - Patient Number 18

A 28 year old woman was referred for chorionic villus sampling by her Obstetrician. The patient herself had read about chorionic villus sampling and DNA analysis of haemophilia, and contacted her Obstetrician with a view to early prenatal diagnosis. In her first pregnancy an amniocentesis had shown a female fetus.

Her male first cousin was affected with Haemophilia A and DNA analysis had been performed by the Haematologists on his mother, and patient number 18's mother.

The family tree had been investigated using a probe for the intragenic polymorphism in the factor VIII gene which gives bands of 1.1 and/or 0.8 kilobases (Kb) with the enzyme Bc1I (Dr I Peake, University of Wales).

The family tree was as follows.



The patient herself had had her factor VIII levels measured (66%) and von Willebrand factor antigen (120%), strongly suggesting she was a carrier. She was heterozygous 1.1/0.8 for the Bc1I polymorphism and so this could be used for analysis.

A high vaginal bacteriology swab showed no growth. Blood group was O Rhesus Positive. At sampling, a single fetus with a crown rump length of 4.3 cms was seen. Placental site was posterior.

Thirty milligrams of villi were obtained on one aspiration. The sample was sent to Dr I R Peake, Principal Scientist, Department of Haematology, University of Wales College of Medicine.

RESULTS

Using a Y specific probe, the fetus was shown to be male. Using the Bc1I polymorphism probe, a 1.1 Kb band alone was seen. Both tests were repeated and gave identical results.

This result strongly suggested that the fetus had inherited Haemophilia A, as the 1.1 band was the allele in her affected cousin.

Termination of pregnancy was carried out at 13 weeks gestation.

Patient Number 48 This 26 year old woman, a carrier for Haemophilia A, was referred via a Consultant Haematologist in another Health Authority. They had tried to organise chorionic villus sampling within their own Health Authority area but had been unsuccessful.

The patient had one brother who was affected with Haemophilia A and one sister who was a carrier. She had had no previous pregnancies. The Director of Derby Haemophilia Centre (who had referred the patient) had applied DNA probes to the family and the patient was fully informative for Haemophilia A.

She was 10 weeks gestation at referral. A high vaginal bacteriology swab was taken and showed no significant growth. On ultrasound, a single fetus, crown rump length 3.8 cms, was seen. Placental site was posterior.

A total of 30 milligrams of villi was obtained and split, such that 20 milligrams were sent to Dr I Peake, Cardiff and 10 milligrams retained for karyotype analysis.

The fetal karyotype was 46,XX.

The patient had an uneventful pregnancy with a spontaneous vertex delivery of a live female, 3.34 kilograms in weight, at 39+ weeks gestation.

(ii) β-Thalassaemia

β-Thalassaemia is caused by at least 30 different mutations (Orkin et al 1983). Most cases of β-thalassaemia have to be diagnosed by linked DNA polymorphisms or by hybridization with oligonucleotide probes. Because most of the polymorphic restriction sites in the β-globin gene cluster occur on both normal and β-thalassaemic chromosomes, it is necessary to establish linkage between a particular restriction fragment length polymorphism (RFLP) and the mutant gene by a family study. This can be done easily if the couple have a normal or homozygous β-thalassaemic child

from which blood can be obtained. Both cases of β-thalassaemia described (patients 42 and 52), had such family studies done (Dr J Old's Laboratory, Oxford). To establish linkage in each case most laboratories study 7 RFLPs, and the presence or absence of each polymorphic site along the chromosomes can be worked out for each family. Examples of this are seen in the description of each case.

Patient Number 42 A 29 year old Pakistani woman was referred for chorionic villus sampling by the Haematology Department, Birmingham Children's Hospital. She had had three previous pregnancies. She had a 9 year old son affected with β-thalassaemia major. She had fetoscopy and fetal blood sampling in her next two pregnancies (1981 and 1983). Both children were found to be unaffected using DNA analysis (Dr J Old, National Haemoglobinopathy Reference Service, Oxford).

High vaginal bacteriology swab was negative. Blood group was O Rhesus Positive. At sampling, a single fetus equivalent in size to 11 weeks gestation was seen. The placental site was anterior.

Fifty milligrams of villi were obtained on one aspiration.

The fetal karyotype was 46,XY.

DNA RESULTS (Dr J Old, Oxford)

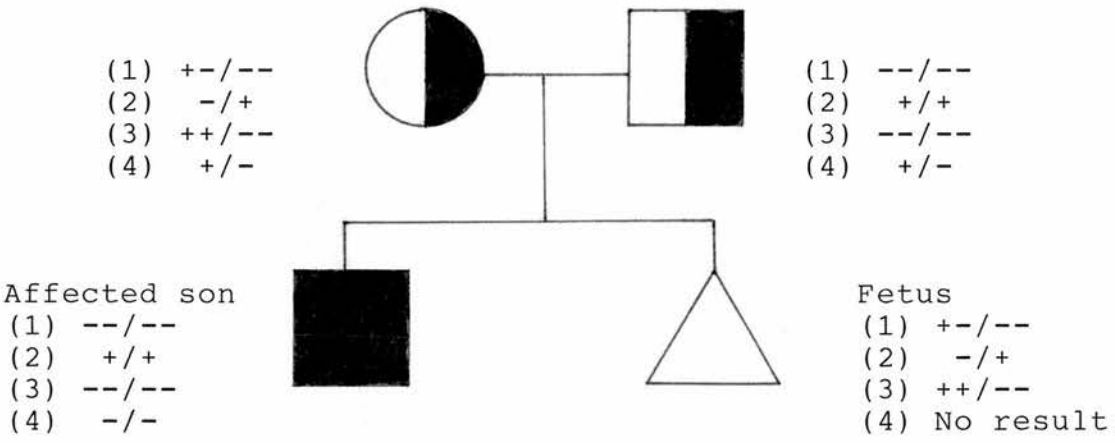
Yield of DNA - 190 µg. Three different DNA polymorphisms indicated that the fetus was either normal or had β-thalassaemia trait.

DNA analysis

Polymorphisms

(1)	Hind III/α	--/+-
(2)	Hind II/ϵ	-/+
(3)	Hind II/$\psi\beta$	--/++
(4)	Hind III/PRK	No result

Linkage analysis



Unfortunately because the DNA was partially degraded when it reached Dr Old's laboratory in Oxford, he could not tell if the fetus had β-thalassaemia trait or not. The probe necessary for that Hind III/PRK 29 produces large bands (18 kilobases) and these had repeatedly not shown up in the fetal sample.

At 39 weeks gestation, the patient had a normal delivery of a live male, 3.25 kilograms in weight. There were no neonatal problems.

Patient Number 52 A 27 year old Cypriot woman, whose husband was also a Cypriot, was referred for chorionic villus sampling by her Obstetrician. Both she and her husband were

β-thalassaemia carriers. She was an insulin dependent diabetic. She had one normal child, (i.e. with normal values of Haemoglobin A_2), born in 1983.
In 1984, she had transcervical chorionic villus sampling performed in London and subsequently spontaneously aborted. Because of this and the fact that she was a diabetic, I felt that the preferred route of sampling was transabdominal.

High vaginal bacteriology swab was negative. Blood group was A Rhesus Positive. At sampling, the placental site was posterior. A single fetus, crown rump length 3.0 cms, equivalent to 9 to 10 weeks gestation was seen.

Transabdominal sampling was performed but only 5 milligrams of villi, insufficient for DNA analysis was obtained. Under antibiotic cover (Metronidazole 200 mgs and Ampicillin 500 mgs), a single transcervical aspiration was performed and a further 40 milligrams of villi were aspirated.

The fetal karyotype was 46,XX.

DNA RESULTS (Dr J Old, Oxford)

Yield of DNA - 85 µg.

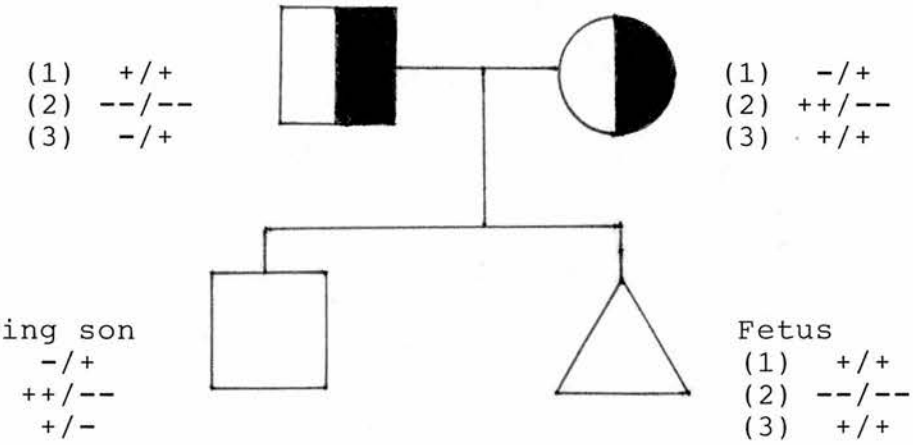
DNA analysis — Polymorphisms

(1) Hind II/ϵ

(2) Hind III/α

(3) Ava/β

Linkage analysis



This showed the fetus to be homozygous for β-thalassaemia. The patient underwent a termination of pregnancy at 13 weeks gestation.

(iii) Sickle Cell Anaemia

The β^S gene responsible for sickle cell anaemia is a single base substitution of A (Adenosine) to T (Thymidine) in Codon 6 of the β globin gene. This base substitution abolishes 3 different restriction enzyme recognition sites in the normal β gene. The third of these, called Mst II generates large fragments that can blot easily and hybridize normally, and this enzyme is now used by most centres for the prenatal diagnosis of sickle cell anaemia (Old 1986).

Patient Number 91 A 36 year old woman, who had two living children with sickle cell disease was referred by the Haematology Department, Birmingham Children's Hospital.

High vaginal swab was negative. Blood group was O Rhesus Positive. At sampling, a single fetus with a crown

rump length of 4.1 cms was seen, equivalent to 11 weeks gestation. Placental site was posterior and right lateral.

Fifteen milligrams of villi were obtained on two aspirations.

The fetal karyotype was 46,XX.

DNA RESULTS (Dr J Old, Oxford)

Yield of DNA - 41 µg.

Mst II/β : -/-

The fetus was homozygous for sickle cell disease.

The patient underwent termination of pregnancy at 13 weeks gestation.

(iv) Cystic Fibrosis

Although Cystic Fibrosis is a disease in which the precise biochemical defects have not been discovered, it can now be detected in many couples at risk using DNA probes linked to the cystic fibrosis locus. The cystic fibrosis gene is now known to be situated on the long arm of chromosome number 7 (at 7q21-q31)[Dean et al 1985; Bartels et al 1986].

For diagnostic purposes, probe pJ3.11, met H and met D are used. The two markers met and pJ3.11 are so close to the cystic fibrosis gene that recombination is likely to be less than 1% (Personal communication quoted by Brock 1987).

On the basis of known allele frequencies, 80% of couples should be fully informative for one of these markers and the remainder to be half informative (Brock 1987).

However, when DNA is not obtainable from an affected child, then prenatal diagnosis using these markers is not possible.

The genotypes of a family may be fully informative when the cystic fibrosis bearing chromosomes in both parents can be identified. The ideal should be that their genotypes are known before pregnancy.

In the 7 patients referred for chorionic villus sampling, 5 were fully informative and 2 were partially informative on the basis of DNA analysis using probes met H, met D and pJ3.11. All patients undergoing sampling had the sample split so that karyotype analysis was also performed as well as DNA analysis. All DNA analysis was carried out by Dr M Super, Clinical Genetics Unit, Royal Manchester Children's Hospital.

Table 44 shows the outcome of the 7 patients.

Patient Number 45 In this case, the family were partially informative using probe met H. The family were fully informative using probe 79a but Dr Super's laboratory were unhappy about using this probe because there would be a 16% error rate for a diagnosis of Cystic Fibrosis using it.

DNA analysis of the fetus showed it to be a homozygote for the met H probe but this meant that the fetus had a 1 in 2 chance of being affected (see Pedigree). After counselling from the Consultant Clinical Geneticist, the patient chose a termination of pregnancy.

TABLE 44

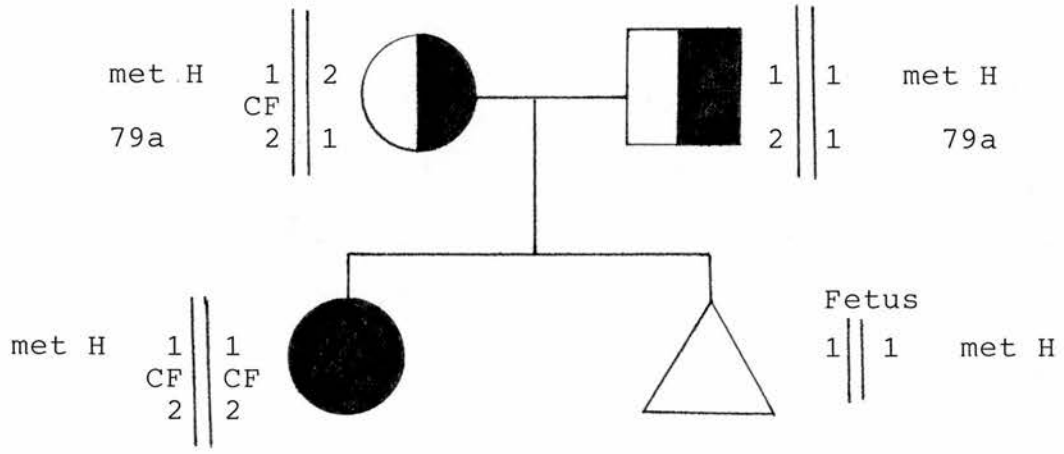
CHORIONIC VILLUS SAMPLING IN FAMILIES WITH CYSTIC FIBROSIS

Patient number	Genotype known before pregnancy	Gestation (weeks)	Prediction	Outcome (birth details, gestation, weight)
36	met H, met D	10	No CF genes 46,XY	Live male, 39 weeks, 3.18 kgs.
45	met H, met D pJ3.11 (partially informative)	10	1:2 risk of CF. 46,XX	T.O.P.
54	met H, met D	10	Carrier 46,XY	Live male, 40+ weeks, 3.07 kgs.
62	met H, met D	10	Carrier 46,XY	Live male, 41+ weeks, 3.59 kgs.
*71	met H, partially informative met D	11+	Carrier 46,XY	Spontaneous abortion at 23 weeks.
95	met H, met D, pJ3.11	10+ & 13	Carrier 46,XY	Live male, 37 weeks, 2.85 kgs.
**98	met H, met D, pJ3.11	10+	No CF genes 46,XY/47,XY +21	T.O.P. Fetus 46,XY

* Further discussed in section 8.8.
** Described in sections 8.3 and 8.6 B(iii).

CF Cystic Fibrosis
kg Kilogram
T.O.P. Termination of pregnancy

Pedigree - patient 45



Patient Number 95 This patient had transcervical chorionic villus sampling performed at 10+ weeks gestation. Twenty milligrams of villi were obtained. The family had been investigated in Edinburgh using probes met H, met D and pJ3.11 and were fully informative. However, due to technical difficulties at the Edinburgh laboratory no result was obtained.

After further counselling by the referring Clinical Genetics Consultant, she underwent transabdominal chorionic villus sampling at 13+ weeks gestation. The placental site was posterior.

Twenty milligrams of villi were obtained on one aspiration. The villi were sent to Dr Super's laboratory in Manchester.

The fetus was found to be a carrier. The fetal karyotype was 46,XY.

She had a spontaneous delivery at 37 weeks gestation of a live male, 2.85 kilograms in weight. There were no neonatal problems.

8.6 COMPLICATIONS OF DIAGNOSTIC CHORIONIC VILLUS SAMPLING

A) Infant Death

Patient number 56 delivered at 27 weeks gestation and the baby died at 2 months of age.

She was a 40 year old woman of Indian origin who wished prenatal diagnosis because of her age. She was randomised to receive chorionic villus sampling after entering the MRC trial. She had had two previous pregnancies resulting in normal deliveries of live female children.

A preliminary high vaginal swab had shown a heavy growth of candida species and she received treatment with Canestan pessaries prior to chorionic villus sampling. Blood group was B Rhesus Negative. At sampling, a single fetus equivalent to 11 to 12 weeks gestation was seen. The placental site was posterior.

Twenty five milligrams of villi were obtained with one transcervical aspiration.

The fetal karyotype was 46,XY.

Immediately following the chorionic villus sampling a Kleihauer estimation was performed showing a feto-maternal transfusion of approximately 5 millilitres. A total of 500 IU of Anti-D was therefore given.

Her pregnancy progressed uneventfully but she went into premature labour at approximately 27 weeks gestation. She was delivered of a live male, 990 grams in weight (50th centile for gestational age and sex). At birth he was ventilated for only a short time.

Clinical progress was satisfactory until 4 to 5 days before death (at the age of 2 months), when he was treated with antibiotics for a Group A Beta Haemolytic Streptococcus which had grown in material from his ear. He rapidly deteriorated after this and three days before his death appeared to have aspirated a large quantity of milk. Despite being put on the ventilator, he could not maintain his partial pressure of oxygen even on 100% oxygen concentrations and he died at 2 months of age.

A final histological report from the Pathologist was as follows:

Cause of death

(1) Bilateral haemorrhagic bronchopneumonia

(2) Aspergillus infection of both lungs

(3) Hypoxic necrosis of the brain.

There was no histological examination of the placenta.

COMMENT

While the chorionic villus sampling procedure was straightforward and atraumatic, a significant fetomaternal haemorrhage occurred and it is interesting to note the presence of a retro-placental clot at delivery. Presumably premature labour occurred because of an antepartum haemorrhage but whether a procedure performed fourteen weeks previously can be implicated in the acute episode is difficult to say without proper histology of the placenta.

This patient and patient number 35 both had significant fetomaternal haemorrhages, as estimated by a Kleihauer test

and both had complications of pregnancy (premature labour at 26 weeks and spontaneous abortion at 13 weeks respectively).

It is possible that the presence of placental clot could lead to a localised inflammatory process leading to necrosis, haemorrhage and further placental separation (D I Rushton, Consultant Pathologist, Birmingham Maternity Hospital - Personal Communication).

B) Mosaicism

In the 100 diagnostic cases, 95 had karyotype analysis performed. There was one true mosaic (patient number 79). All karyotype analysis was on direct chromosome preparations as outlined in chapter 7.

Table 45 summarises the cases of Mosaicism.

Patient Number 79 A 36 year old woman had transcervical chorionic villus sampling performed at 11 weeks gestation. The indication was maternal age. The placental site was anterior and 40 milligrams of villi were aspirated.

Twenty five metaphase spreads were analysed. Seven spreads showed a 46,XX karyotype and 18 spreads a 45,X karyotype.

Amniocentesis was performed and 30 metaphase spreads analysed in culture. Twelve spreads showed 46,XX and 18 showed 45,X.

After termination of pregnancy, culture of fetal skin showed a 46,XX/45,X karyotype.

TABLE 45

MOSAICISM

PATIENT NUMBER	INDICATION	CVS RESULT	AMNIOCENTESIS	PREGNANCY OUTCOME
10	Age	46XY/47XY,+15 [40/4]	46,XY	Term male
39	Prev.Downs	46XY/47XY,+3 [61/2]	46,XY	Term male
53	Age	46XY/47XY,+3 [36/4]	-	Term male
79	Age	46XX/45X [7/18]	46XX/45X [12/18]	T.O.P.
98	C.F.	46XY/47XY,+21 [24/8]	-	T.O.P. 46XY fetal culture
100	Prev.Downs	46XY/47XY,+3 [28/2]	-	Term male

T.O.P. Termination of Pregnancy

There were five cases of mosaicism apparently confined to the placenta.

(i) Trisomy 15

Patient Number 10 A 38 year old woman had chorionic villus sampling for maternal age reasons. Transcervical chorionic villus sampling was performed at 11 weeks gestation. The placental site was anterior, 20 milligrams of villi were aspirated.

A total of 44 metaphase spreads were analysed. Forty spreads showed a 46,XY karyotype and 4 spreads a 47,XY,+15 karyotype.

Although the significance of this was in doubt, after counselling from the Clinical Genetics Consultant, the patient underwent amniocentesis at 16 weeks gestation. The karyotype of all cultured cells was 46,XY. At 40 weeks gestation, she had a normal delivery of a live male, 3.84 kilograms in weight. There were no neonatal problems.

(ii) Trisomy 3

Three patients numbered 39, 53 and 100 had Trisomy 3.

Patient Number 39 A 27 year old woman, who had had a previous Down's baby, underwent transcervical chorionic villus sampling between 10 and 11 weeks gestation. The placental position was posterior and 30 milligrams of villi were aspirated.

A total of 63 metaphase spreads were examined. Sixty-one showed a 46,XY karyotype and 2 showed a 47,XY,+3 karyotype.

Amniocentesis was performed at 17 weeks gestation. All cultured cells showed a 46,XY karyotype. At term, she had a normal delivery of a live male, 3.85 kilograms in weight. There were no neonatal problems.

Patient Number 53 A 41 year old woman underwent transcervical chorionic villus sampling between 10 and 11 weeks gestation. The placental site was posterior and 10 milligrams of villi were obtained.

A total of 40 metaphase spreads were examined. Thirty-six showed a 46,XY karyotype and 4 showed a 47,XY,+3 karyotype.

Her referring Obstetrician did not recommend to her that any further investigation be carried out as he did not regard the result as being indicative of fetal abnormality. She had a normal delivery at term of a live male, 3.09 kilograms in weight. There were no neonatal problems.

Patient Number 100 A 38 year old woman had transcervical chorionic villus sampling performed at 11 weeks gestation. She had had a previous child with Down's Syndrome. The placental site was posterior and thirty milligrams of villi were aspirated. Thirty metaphase spreads were examined. Twenty eight had a 46,XY karyotype and 2 had a 47,XY,+3 karyotype.

No further antenatal tests were recommended.

She was delivered by Caesarean Section at 40 weeks gestation, of a normal male, 4.3 kilograms in weight. There were no neonatal problems.

(iii) Trisomy 21

Patient Number 98 This 23 year old woman, with a child with Cystic Fibrosis, had transabdominal chorionic villus sampling performed between 10 and 11 weeks gestation. The placental site was fundal and posterior. A total of 30 milligrams of villi was obtained (DNA analysis - see section 8.3).

Karyotype analysis was also performed. A total of 32 metaphase spreads were examined. Twenty four had a 46,XY karyotype and 8 had a 47,XY,+21 karyotype.

The Clinical Geneticist could not give the patient and her husband complete reassurance that the fetus did not have Mosaic Trisomy 21 in other cell lines. As the couple had undergone prenatal diagnosis to minimise the risk of having a child with an abnormality, they felt that they would prefer the earlier option of termination of pregnancy, rather than wait for later confirmatory tests.

She underwent termination of pregnancy at twelve weeks gestation. Culture of the fetal skin (6 cells analysed and 95 cells counted) showed a 46,XY karyotype with no evidence of the abnormal Trisomy 21 cell line.

(iv) Discussion

Mosaicism is defined as the presence within one individual of two or more cell lines exhibiting different karyotypes. Pseudo-mosaicism, in which the two cell lines are present in the karyotype of the cultured tissue but not within the fetus, has been described in amniotic fluid cell cultures. It is thought to be due to either a cultural artifact occurring in vitro or contamination of the amniotic

fluid sample by chorionic or maternal tissues.

In a review of mosaicism in amniotic fluid culture, Gosden (1983) reported that 80% of autosomal mosaicism was due to pseudo-mosaicism but that pseudo-mosaicism only accounted for 40% of sex chromosome mosaicism.

The mosaicism occurring in the five cases in which it was not confirmed in the fetus could not be due to cultural artifact as all karyotype were direct preparations. In a series of 46 third trimester placentae that were karyotyped, two were found to have mosaicism confined to the placenta (Kalousek et al 1983). These authors and others (Gosden 1983; Cheung et al 1987), have speculated that mosaicism may occur more frequently in the chorion and have quoted the findings of a study of the mouse blastocyst in which only three out of its 64 cells formed the embryo, the remaining cells forming the trophoblast (49 cells) and amnion, yolk sac and allantois (12 cells)(Markert et al 1978). The fact that not all the cells of the extraembryonic tissues are of fetal origin could mean that non-disjunction during early embryogenesis might produce mosaicism within the placenta or fetus but not necessarily in both.

In a review of 6125 karyotype analyses from diagnostic chorionic villus samples, Mikkelsen reported 45 mosaics of which 37 could not be confirmed in fetal cells, amniotic fluid culture or at birth (Mikkelsen 1987).

The mosaicism involving the sex chromosome in case number 79, warranted further tests which confirmed the results from the villus sample. In mosaicism involving the sex chromosomes detected in villus samples, follow up

investigations should be recommended. Similarly, in the case of mosaicism for Trisomy 21 (case number 98), which is known to account for 1-2% of individuals with Down's Syndrome (Gosden 1983), further tests would be indicated assuming the parents did not wish immediate termination of pregnancy. In cases in which mosaicism involves outcomes of no known clinical significance e.g. Trisomy 3 or 15, further prenatal tests should not be recommended especially, as in this series, the number of metaphases exhibiting the trisomy was far outnumbered by the number with a normal karyotype.

The implications for counselling patients who wish chorionic villus sampling for karyotype analysis is that a discrepancy between the karyotype of the placenta and that of the fetus may occur in up to 5% of cases. Where this discrepancy involves a sex chromosome, chromosomes 21, 18, 13 or 8, then further prenatal diagnostic tests should be performed. Where, as is more likely, it involves chromosomes of no known clinical significance e.g. Trisomy 3 or 15, then further prenatal tests are not warranted.

8.7 OUTCOME OF 100 DIAGNOSTIC CHORIONIC VILLUS SAMPLING PROCEDURES

The outcome of the one hundred diagnostic chorionic villus sampling procedures is shown in figure 20.

A) Premature Delivery

Delivery before thirty seven weeks gestation occurred in six patients. Three of these have already been described Patient 56, section 8.6 A; Patient 72, section 8.5 A and Patient 74, section 8.4 A.

Of the remaining three patients delivery occurred in the following circumstances.

Patient Number 32 This patient had transcervical chorionic villus sampling performed at 10½ weeks gestation for fetal sexing. The sex was female.

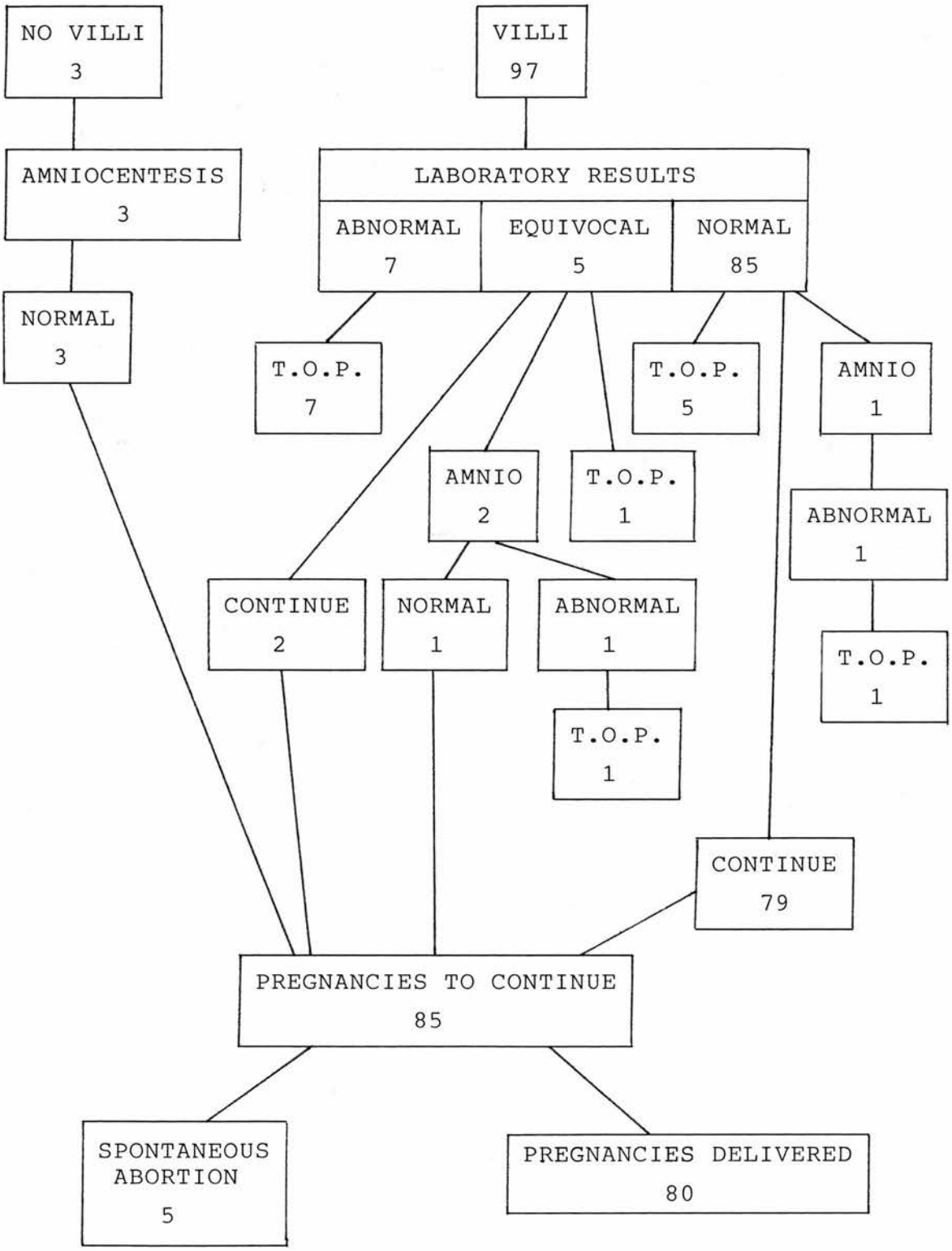
At 36 weeks gestation, labour was induced because of suspected intrauterine growth retardation. A vaginal delivery of a live female, 2.07 kilograms in weight (below the 10th centile for gestational age and sex), occurred. There were no neonatal complications.

Patient Number 40 This 42 year old woman had transcervical chorionic villus sampling because of maternal age. A normal karyotype was obtained.

She went into spontaneous labour at 35 weeks gestation and had a vaginal delivery of a live male, 2.66 kilograms in weight (between the 50th and 90th centile for gestational age and sex). There were no neonatal complications.

FIGURE 20

OUTCOME OF 100 DIAGNOSTIC CHORIONIC VILLUS SAMPLING PROCEDURES



T.O.P. Termination of pregnancy
AMNIO Amniocentesis

Patient Number 66 This 39 year old woman had transcervical chorionic villus sampling at 10 weeks gestation for maternal age reasons. The placental site was posterior and low.

She had an emergency Caesarean Section at 35 weeks and 3 days gestation because of vaginal bleeding. Placenta praevia was diagnosed. A live male, 2.15 kilograms in weight was delivered (between 10th and 50th centile for gestational age and sex).

B) Birth Weights of the 80 Delivered Patients

The birth weights of delivered patients were recorded and compared to standard charts, of weight related to gestational age and sex, devised by Gairdner and Pearson 1971 (revised 1985).

The mean birth weight of the eighty patients was 3.38 kilograms, S.D. 0.61.

Table 46 shows the birth weights by centiles and in four cases the birth weight was below the 10th centile. There were no neonatal complications in any of these cases.

TABLE 46

BIRTH WEIGHT CENTILES (after Gairdner and Pearson 1971)

Birth Weight Centiles	Number of Babies
< 10th	4
> 10th ⩽ 50th	31
> 50th ⩽ 90th	39
> 90th	6
Total	80

8.8 SPONTANEOUS ABORTION

Spontaneous abortion in this series is defined as the death and expulsion of the fetus before 28 weeks gestation. Five patients had a spontaneous abortion following chorionic villus sampling. They are detailed individually and Table 47 summarises the cases.

Patient Number 27 A 39 year old woman was referred for chorionic villus sampling by her Obstetrician. She had two normal deliveries and one extra amniotic Prostaglandin termination of pregnancy at 22 weeks gestation after amniocentesis had shown a fetus with Down's Syndrome.

High vaginal bacteriology swab showed no growth. Blood group was O Rhesus Positive. At sampling, a single fetus, 11 weeks gestation in size, was seen. The placental position was posterior and lateral.

Two transcervical aspirations produced less than 10 milligrams of villi and a third about 10 milligrams of villi. There was some slight bleeding after the procedure.

The fetal karyotype was 46,XY.

Vaginal bleeding persisted and she was admitted to hospital at 12 weeks gestation. The day prior to her spontaneous abortion a live fetus was seen on ultrasound scan. She aborted at 13 weeks gestation.

Histology of abortus The Pathologist's report was as follows:-

An intact amniotic sac containing a fresh externally normal male fetus crown rump length 7.4 cms, crown heel 9.8 cms, foot 1.1 cms weighing 24.9 grams. The cord

TABLE 47

SPONTANEOUS ABORTIONS FOLLOWING CHORIONIC VILLUS SAMPLING

Patient number	Gestation at CVS (weeks)	CVS attempts	Placental site	Villus weight (mgs)	Gestation at spontaneous abortion (weeks)
27	11	3	Posterior	20	13-14
35	11	3	Post/Fundal	5	13
59	11	1	Posterior	15	15
71	11+	1	Posterior	30	23
73	11	1	Anterior	15	13-14

measures 11 cms. The placenta is fragmented and received with much fresh blood clot. No lesion attributable to chorionic villus biopsy is identified though this does not exclude the possibility that the procedure was a contributory factor leading to abortion.

Histology

The cord, membranes and placenta are normal.

Patient Number 35 A 36 year old woman, who had had one previous normal child by her first marriage, wished prenatal diagnosis because of maternal age. She was randomised to receive chorionic villus sampling.

High vaginal swab showed no growth. Blood group was O Rhesus Negative. At sampling, a single fetus equivalent in size to 10 weeks gestation was seen. The uterus was retroverted. The placental site was fundally placed and transcervical chorionic villus sampling was difficult because of this.

Three attempts were made and 5 milligrams of villi were obtained from the edge of the placenta.

The fetal karyotype was 46,XY.

Some slight vaginal bleeding was noted at the time of the procedure. As she was Rhesus Negative, a Kleihauer test was done after the procedure and this showed an approximate 25 millilitres estimate of fetomaternal transfusion. A total of 2500 IU of Anti-D was therefore required.

Twelve days later, she aborted spontaneously. Unfortunately, no histological examination of the placenta or fetus was carried out at her local hospital.

Patient Number 59 A 42 year old woman was referred for chorionic villus sampling by her Obstetrician. She had had one normal delivery of a healthy female and two first trimester miscarriages at 7 and 9-10 weeks gestation respectively. She entered the MRC trial and was randomised to receive chorionic villus sampling.

Preliminary high vaginal swab was negative. Blood group was O Rhesus Positive. An ultrasound scan at her local hospital had suggested twins, but at sampling a single fetus only was seen with a size equivalent to 11 weeks gestation. There was no evidence of a second sac. Placental site was posterior.

One attempt at transcervical chorionic villus sampling produced 15 milligrams of villi. There were no difficulties.

The fetal karyotype was 46,XX.

She was admitted to her local hospital at 14+ weeks gestation with a threatened miscarriage. Ultrasound scan was performed which showed a live fetus. She miscarried two days later at 15 weeks gestation. Histology was carried out at her local hospital and the Pathologist's report was as follows:-

Specimen: Fetus and placenta

Apparently normal male fetus 9.5 cms crown to rump length. Head circumference 9.5 cms and weighing 41 grams. It is not macerated and the organs show no major abnormality. Separate placenta and membranes weighing 63 grams. There is a velamentous insertion of the cord. The placenta appears normal.

Histology

Placenta and fetal organs showed no histologic abnormality.

Patient Number 71 A 32 year old woman was referred for chorionic villus sampling by the Clinical Genetics Department. She had one normal son and one son affected with Cystic Fibrosis.

The DNA probes on the affected son and the parents showed that they were partially informative using probe met D and uninformative using probe met H (see section 8.5 for description of DNA probes on Cystic Fibrosis).

Preliminary high vaginal swab showed a growth of Gardnerella Vaginalis and pre, per and post procedure treatment with metronidazole, 200 milligrams three times a day, was instituted. Blood group was A Rhesus Positive. At sampling, a single fetus with a gestational age equivalent to 11 weeks was seen. Placental site was posterior. Thirty milligrams of villi were obtained on one transcervical aspiration.

The fetal karyotype was 46,XY. DNA studies showed the fetus to have the genotype "1,1" meaning that it was either a carrier or unaffected.

She was admitted to her local hospital at 23 weeks gestation and miscarried.

Histology of abortus

A male fetus measuring 165 mms crown rump length appearing to be normal on external examination. Dissection shows no obvious abnormality of the internal organs. The placenta weighs 168 grams and it is entire with little infarction. Sections show normal placental tissue only.

Patient Number 73 A 39 year old woman, who had had two normal deliveries and one miscarriage at 8 weeks gestation, was randomised to chorionic villus sampling after entering the MRC trial.

High vaginal swab showed no growth. Blood group was O Rhesus Positive. At sampling, a single fetus was seen with a gestational age of 10-11 weeks. The placental site was anterior.

Fifteen milligrams of villi were obtained with one transcervical aspiration.

The fetal karyotype was 46,XX.

She had an ultrasound scan performed two weeks later showing a live fetus of appropriate size. She miscarried the following day at home. Only a fetus was available for histology report. The Pathologist's report was as follows:-

Histology of fetus

Macroscopic: Small fetus showing early maceration. Crown rump length 8 cms, crown-heel 10.8 cms, foot length 1.1 cms, 6 cms of cord on body. The external genitalia are ambiguous but no external abnormality evident. The internal genitalia are female, and no definite internal abnormality is seen.

Histology

The measurements correspond by size to a gestation of 13 weeks. Sections of the fetal organs show no diagnostic abnormality. The umbilical cord contains three vessels.

Diagnosis

Macerated female fetus showing no external or internal congenital malformations.

DISCUSSION

The spontaneous abortion rate in this diagnostic series of 100 patients is 5.7%. This is calculated after subtraction of the 15 patients who had termination of pregnancy performed. This rate compares to the 1% loss rate following amniocentesis (Tabor et al 1986).

The abortion rate in this series of patients can be compared to the varying rates of spontaneous abortion, following transcervical chorionic villus sampling, of 1.2% to 8.5% quoted by those centres reporting on series in excess of 100 patients (Jackson 1987).

The critical question, what is the inherent miscarriage rate due to chorionic villus sampling, is difficult to answer. Within this series of patients, four of the five who miscarried had histological examination of the abortus performed and none had abnormalities that the Pathologist attributed to the sampling procedure. My own belief is that patients 27 and 35, who had persistent bleeding after the procedure, and evidence of considerable fetomaternal haemorrhage, aborted as a direct result of the procedure. Both cases required three passages of the cannula to acquire a diagnostic sample. The other 3 patients, all of whom had had previous spontaneous abortions and in whom there was no histological evidence of a direct link to chorionic villus sampling, may fall into the category of unrelated spontaneous abortion. The effect of a history of previous spontaneous abortion on the risk of subsequent abortion will be examined in the next chapter.

The role of infection in spontaneous abortion is well known. Transcervical chorionic villus sampling introduces the possibility of infecting the sterile intrauterine environment. In all 100 diagnostic cases, care was taken to exclude or treat infection prior to sampling as well as administer a prophylactic antibiotic to decrease the risk of abortion due to anaerobic or similar organisms, known to be difficult to routinely culture. In order to establish whether or not infection, and thus inflammation, of the abortus is related to chorionic villus sampling, it would be necessary to demonstrate that the proportion of cases that abort, showing signs of infection, is significantly greater than that in a control population of spontaneously aborting women of similar gestation. More background data relating to the true incidence of spontaneous abortion in ultrasonically intact pregnancies followed from the first trimester to 28 weeks gestation is needed. This will be examined in the next chapter.

Proper histological examination of abortions following chorionic villus sampling is mandatory. Pathological findings of decidual inflammation and chorioamnionitis in abortuses following chorionic villus sampling should be interpreted with caution. Chorioamnionitis is found in at least a quarter of fresh spontaneous abortions (Rushton 1984) and thus, in individual cases, it cannot be assumed that the chorionic villus sampling was responsible for its presence.

The true excess miscarriage rate attributable to chorionic villus sampling can only be accurately assessed

in large scale randomised trials (Chalmers 1987) in which matched patients are randomly assigned to receive either amniocentesis or chorionic villus sampling at an early stage in their pregnancy and followed through to delivery.

The virtues of one method of chorionic villus sampling compared with another can again only be assessed in a similar fashion. According to Jackson (Jackson 1987), in those centres reporting their experience of transabdominal procedures, the fetal loss rate varies from 1.7% to 4.8% (compared with 1.2% to 8.5% for the larger series of transcervical procedures).

AN INVESTIGATION OF SPONTANEOUS ABORTION IN ULTRASONICALLY VIABLE PREGNANCIES

9.1 INTRODUCTION

In the diagnostic series of 100 pregnancies in which chorionic villus sampling was performed (Chapter 8), there were five spontaneous abortions. This represents a spontaneous abortion rate of 5.8% for the diagnostic series (5 of 85 pregnancies intended to continue). Without knowing the background rate of naturally occurring spontaneous abortion, the excess abortion rate due to chorionic villus sampling is difficult to quantify.

Estimations of the naturally occurring spontaneous abortion rate are imprecise and there is little reliable actuarial data available for determining the chances of any given pregnancy ending in spontaneous abortion. Such information would be invaluable in counselling women prior to procedures such as chorionic villus sampling. Biochemical, clinical and recently ultrasound studies have all produced different estimations of fetal loss.

In biochemical studies in which pregnancy is diagnosed by a positive beta-human chorionic gonadotrophin (beta HCG) assay, the diagnosis of pregnancy and the subsequently derived determination of fetal loss is dependent on the characteristics of the assay used. This was demonstrated by the studies of Miller *et al* 1980, Edmonds *et al* 1982 and Whittaker *et al* 1983, in which the spontaneous abortion rates in biochemically diagnosed pregnancies that were subsequently recognised clinically were 13.7%, 11.7% and 12.9% respectively. The

unrecognised loss rates (spontaneous abortion rates in so-called occult pregnancies) were 32.9%, 56.8% and 7.6% respectively. The disparity in the latter figures reflects the different assays and criteria used for the biochemical diagnosis of pregnancy in each study.

Clinical studies, both retrospective and prospective, are flawed by the difficulty in determining the significance of a delayed or missed period, which could represent either an early spontaneous abortion or an irregularity of the menstrual cycle. Most retrospective studies (reviewed by Simpson and Mills 1986) consistently report clinical spontaneous abortion rates of between 12 and 15%. Recent retrospective data (Wilson et al 1986; Christiens et al 1986; Gustavii 1984 b and Gilmore et al 1985) examined those pregnancies in which fetal viability was assessed ultrasonically. These studies all showed a much lower spontaneous abortion rate than that quoted from clinically recognised abortion studies. However, all the recent ultrasound studies were retrospective and in one widely quoted study (Wilson et al 1986), the indication for first trimester ultrasound assessment was vaginal bleeding in up to 27% of their patient population. Therefore their data may not be relevant to estimations of background risks in populations undergoing chorionic villus sampling.

A prospective series of patients was therefore examined in whom there was ultrasound evidence of fetal viability prior to twelve weeks gestation and who were followed up to at least twenty eight weeks gestation.

9.2 PATIENTS AND METHODS

All patients presenting to the Birmingham Maternity Hospital for their first ante natal visit have an ultrasound scan performed. Five hundred patients were selected in whom a live fetus was seen, with a gestational age as assessed by crown rump length measurements of less than twelve weeks gestation. The names, Medical Record number and parity of these patients were recorded. Patients who presented at their first visit in excess of twelve weeks were excluded, as were multiple gestations and those in which there was no or doubtful evidence of fetal viability. The Medical Records of the five hundred patients were then examined after the five-hundredth recruited patient was at least 28 weeks gestation.

9.3 RESULTS

Table 48 shows the age group distribution of the 500 patients. There were ten spontaneous abortions (2%) and four induced abortions. Of these four, one was performed on mental health grounds, one for a complication of Rhesus disease at 23 weeks gestation and two were for neural tube defects at 20 and 22 weeks gestation.

Table 49 lists the gestation at booking, as assessed by ultrasound, and the gestation at abortion in the 10 patients who spontaneously aborted. One patient had spontaneous rupture of the membranes at 19 weeks gestation. The fetus was 46,XX at autopsy and structurally normal. One patient developed hydramnios at 21 weeks gestation and premature labour. The fetus was anatomically normal at autopsy. Two patients had vaginal bleeding and aborted at 14 weeks gestation.

TABLE 48

AGE DISTRIBUTION OF PATIENTS

	Spontaneous abortion		Totals	
Age group (yr)	N	%	N	%
0-15	0	0.0	1	0.2
16-20	1	3.3	30	6.0
21-25	2	1.6	122	24.4
26-30	6	3.1	195	39.0
31-35	1	0.9	116	23.2
36-40	0	0.0	32	6.4
41+	0	0.0	4	0.8
Total	10		500	

N Number

TABLE 49

GESTATIONAL AGE AT BOOKING SCAN AND ABORTION

Gestation at scan (week)	Gestation at abortion (week)
8	12
9+	10
9+	12
10	21*
8+	11
11+	14
10	19**
9+	14
8+	10
11	12

* Hydramnios and premature labour. No fetal abnormality at autopsy.

** Spontaneous rupture of membranes at 19 weeks. No fetal abnormality at autopsy.

Table 50 shows the abortion rate in those patients with no history of abortion and in those with a history of one or more spontaneous abortions. Ten patients with a history of therapeutic induced abortion were excluded. There was a highly significant difference in the spontaneous abortion rate, from 0.6% in patients with no history of spontaneous abortion to 5.5% in those with such a history ($p<.005$).

Table 51 shows the number of patients with a viable fetus at specific gestational ages and the number of subsequent spontaneous abortions. There were six spontaneous abortions out of 157 women in the group below ten weeks gestation (3.8%), compared with four out of 343 women (1.2%) at greater than ten weeks gestation at the initial booking scan. These differences are significant ($X^2 = 3.87$; $.05>p>.001$).

9.4 DISCUSSION

The 'naturally occurring' spontaneous abortion rate in this group of prospectively studied women was 2%. Recent ultrasound studies have shown similar figures (Wilson *et al* 1986; Christiens *et al* 1984 and Gilmore *et al* 1985). If this is taken as the minimum figure then for the diagnostic series of chorionic villus sampling procedures, the excess abortion rate is at most 3.5%.

This study has also shown that other factors must be considered when estimating the risk of spontaneous abortion. One factor is a history of spontaneous abortion which in this study increased tenfold the risk of aborting compared with the group of women who had never had a spontaneous abortion. In the diagnostic series, two of the five cases who aborted following

TABLE 50

HISTORY OF PREVIOUS SPONTANEOUS ABORTIONS

	No. of patients	Spontaneous abortions	
		N	%
No history	339	2	0.6
One or more abortions	147	8	5.5*

* $X^2 = 11.9$; $p<.005$

TABLE 51

GESTATIONAL AGE AT BOOKING SCAN

Age (wk)	No. of patients	No. of spontaneous abortions
<8	16	0
8-9	45	3
9-10	96	3
10-11	152	2
11-12	191	2

chorionic villus sampling had had previous first trimester spontaneous abortions.

Spontaneous abortion is less likely as pregnancy progresses, Gustavii 1984 b. This study confirms this trend. Spontaneous abortion at less than ten weeks gestation was up to three times greater than that at over ten weeks gestation. This has implications for the timing of chorionic villus sampling and, in my opinion, chorionic villus sampling should not be offered below ten weeks gestation, as the chance of aborting is higher. Comparisons of loss rates from different centres due to chorionic villus sampling should take into account the gestation at which sampling occurred.

Previous reports (Gustavii 1984 b; Gilmore et al 1985) have already demonstrated the adverse effects of maternal age on spontaneous abortion rates but the number of patients in this prospective study aged 35 or older was too small to draw any conclusions about this factor. Four of the five spontaneous abortion cases from the diagnostic series of chorionic villus sampling procedures were over 35 years of age.

A large prospective study of spontaneous abortion in women in the older age group would be needed to estimate a background rate of spontaneous abortion, but because of the small number of older women in any one centre, a multi-centre study would be needed.

APPENDIX 1

PATIENT	CANNULAE	
1	AL	AL
2	MSS	AL
3	MSS	P
4	MSS	AL
5	P	AL
6	P	MSS
7	MSS	MSS
8	P	AL
9	AL	AL
10	AL	AL
11	P	MSS
12	AL	P
13	MSS	MSS
14	AL	P
15	MSS	AL
16	P	MSS
17	MSS	MSS
18	AL	AL
19	AL	AL
20	P	P
21	MSS	P
22	P	MSS
23	AL	P
24	MSS	AL
25	MSS	MSS
26	P	P
27	AL	AL
28	MSS	P
29	MSS	P
30	MSS	AL
31	P	MSS
32	AL	P
33	P	P
34	MSS	MSS
35	MSS	P
36	P	MSS
37	AL	P
38	MSS	MSS
39	P	MSS
40	AL	P
41	MSS	MSS
42	MSS	AL
43	P	MSS
44	MSS	MSS
45	MSS	P
46	AL	AL
47	AL	AL
48	P	P
49	P	MSS
50	P	AL

Generation of orders: 1 AL Aluminium
2 MSS Malleable Stainless Steel
3 P Portex

APPENDIX 2

DATA SHEET

NAME

RESEARCH NO.

AGE

BLOOD GROUP
KLEIHAUER Pre-

L.M.P.

Anti-D(Yes/No)

GESTATION DATES.

H.V.S.

U.S.S.

C.R.L.
F.H.
Placental Site
Cannula Seen
Good/Poor/N.S.

Ex. INSTRUMENT

DATE: TIME:
Patient Reaction
Pain 1 2 3
Bleeding 1 2 3

Stainless Steel
Aluminium
Portex

Insertion Difficulty
1 2 3
No. Insertions

LABORATORY USED

INSTRUMENT USED:

B.M.H.

S.S.
AL
POR

E.B.H.

S.S.
AL
POR

C.O.P.

Date:
Time:
U.S.S.
F.H.
Comments:

CYTOGENETICS

	E.B.H.	B.M.H.
Transport Medium	F10/CHANG	F10/CHANG
Adequate Villi	YES/NO	YES/NO
Villi Wt.		
Direct Prep. (result)	46XX/46XY/_____	46XX/46XY/_____

Time to Result ( ) hours
Tissue Culture (result)

APPENDIX 3

GIEMSA-banding (G-banding)

The method used is that of Seabright (Seabright 1971)

1. Aged slides or slides aged overnight at 50°C, are used.
2. Standard Salt Concentrate (SSC) is made from Concentrated Salt Concentrate (CSC).
3. Unstained slides are put into SSC for half an hour.
4. Slides are next put into 0.9% Saline at room temperature to cool.
5. Slides are put into 0.6 mls of bactotrypsin in 60 mls of 0.9% Saline for 1 minute. This step bands the chromosomes.
6. Slides are washed first in 0.9% Saline.
7. Further wash in Phosphate Buffer Solution (PBS). This step eliminates crystal formation on the slides.
8. Slides are stained with freshly prepared Leighman stain, in a concentration of 1:5 with PBS. Staining takes approximately 5 minutes.
9. Slides are washed in PBS and blotted dry.

APPENDIX 4

CHORIONIC VILLUS SAMPLING PACK STERILE

No.	Description
1	Wool balls sterile
1	Swab gauze 10 cm x 10 cm (5's) sterile
2	Towel dressing folded 16 x 16 in unsterile
2	Linen work drape (202) type
2	Gallipots foil 100 ml unsterile
1	Tray wrap blue 1400 mm x 1000 mm
1	Cusco's speculum
1	Tenaculum
2	Sponge holding forceps

APPENDIX 5

PUBLICATIONS FROM THIS THESIS

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